AUTOXIDATION OF HYDROCARBONS AND POLYOLEFINS

Kinetics and Mechanisms

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This book endeavors to en
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dation scheme of hydrocarbons
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(2)

H + R· (3)

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lucts + O_2 (6)

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This was observed by Elley (*7*)
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not oxidize to any extent under
-1 shows the time required for
d of 1,4-naphthohydroquinone

TABLE 3-1

Evidence for the Interaction of Phenolic Antioxidants with Oxygen (*5*)

Antioxidants	[AH], moles/mole ester × 10^4	[ROOH], moles/mole ester × 10^2	p, mm	t_∞ min
1,4-Naphtho-hydroquinone	0.76	17.7	10	100
	0.77	17.7	25	50
	0.80	17.7	50	32
Hydroquinone	0.80	17.7	50	105

in a system containing ethyl linoleate and its hydroperoxide. It is noted that the 1,4-naphthohydroquinone is removed from the system more rapidly than hydroquinone and, in contrast to hydroquinone, the rate of removal increases with increasing oxygen pressure. Some other evidence for reaction (7) was reported by Shelton and co-workers (*8*). Thus, in rubber oxidation, certain isotopic effects occurred in a reversed order (see Section II) and could be accounted for by assuming that the antioxidant was also involved in the initiation reaction.

Under some conditions, the products of reaction (7) may involve the hydroperoxide of the inhibitor (*9,10*), but under others extensive rearrangements may occur (*11*). Although oxidation will, in general, reduce inhibitor efficiency, in certain cases the products may be more efficient than the original inhibitor. This can lead to an increase in overall efficiency as the inhibitor is oxidized (*12*).

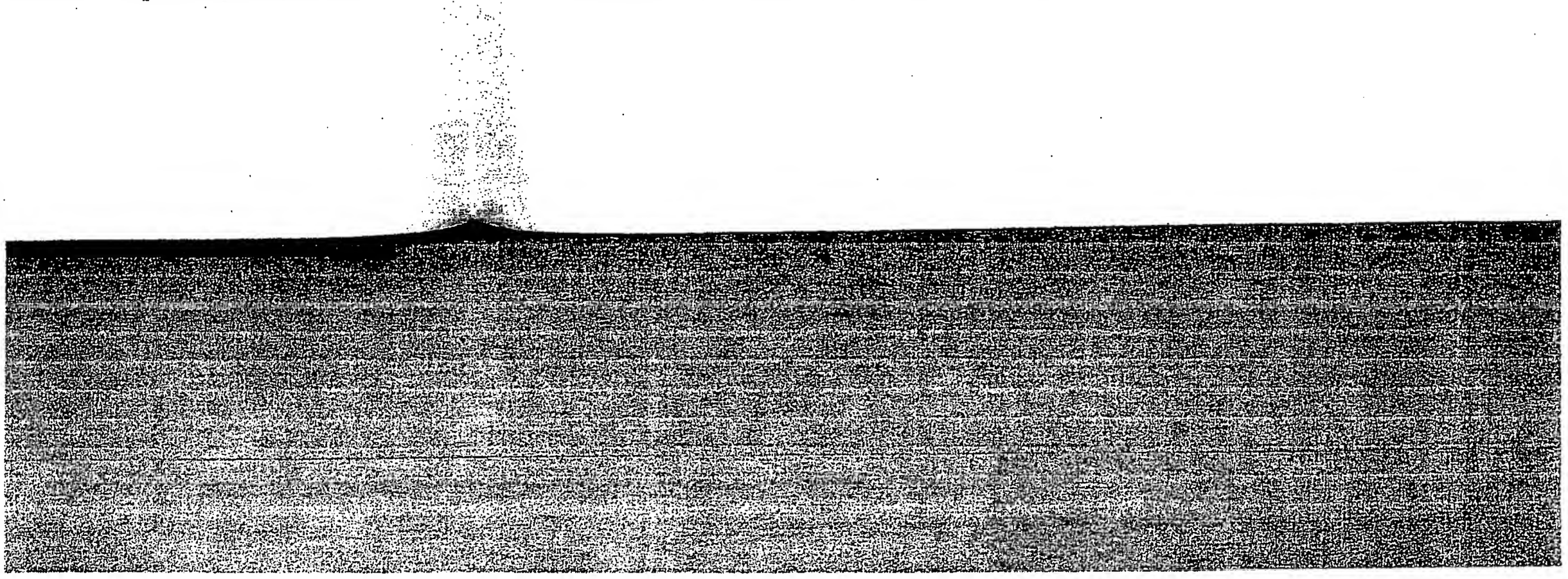
B. Participation in Hydroperoxide Decomposition

1. *Sulfur-Containing Antioxidants*

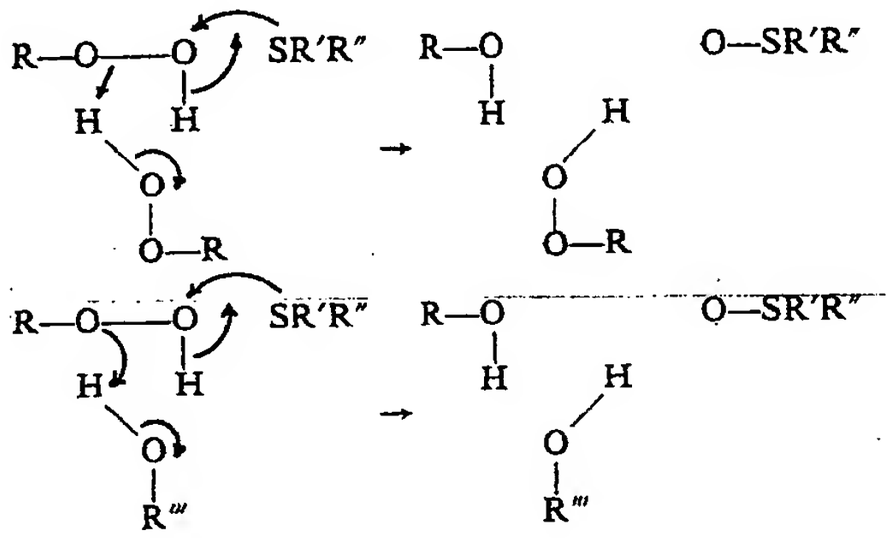
The interaction of hydroperoxides with monosulfides may be represented by (*13,14*)

$$R_2S + RO_2H \rightarrow R_2SO + ROH \quad (8)$$

There is reason to believe that selenides, tertiary amines, phosphines, and phosphites behave similarly as the sulfides. These compounds should be capable of decomposing hydroperoxides without the formation of free-radical intermediates, and thus function as inhibitors. However, the reaction of saturated sulfides with hydroperoxides is more complex than indicated by reaction (8). Thus, the reaction between saturated sulfides



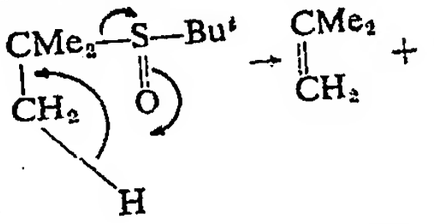
and *t*-butyl hydroperoxide is bimolecular with respect to hydroperoxide concentration in hydrocarbon solvents but unimolecular in alcoholic solvents (*13*,*14*). Two mechanisms have been postulated to account for these results:



(9)

(10)

Although reactions (9) and (10) involve direct hydroperoxide decomposition by the sulfides, evidence exists supporting the premise that the sulfides above are not necessarily the true or active inhibitor. In fact, it has been reported that the sulfides showed inhibitory power only after a small amount of oxygen had been absorbed (*15*). This implied that the sulfides by themselves are not the active or primary inhibitors but rather one or more of their oxygenated derivatives produced in the autoxidizing medium. These derivatives could be sulfoxides, sulfones, thiolsulfinates, thiolsulfonates, or disulfones derived from sulfide inhibitors. Upon decomposition of these derivatives, e.g., thiolsulfonates, sulfonic acids could be formed, hence initiating hydroperoxide decomposition. The thiolsulfonates and the sulfoxides were found to be active inhibitors. Thus, when added directly to an autoxidizing system they showed inhibitory effects at much lower concentrations than the parent sulfides without any initial oxygen uptake by the system. Further, their retardation effect was comparable to conventional antioxidants such as phenyl-β-naphthylamine (*15*). Active sulfoxide inhibitors may be produced by interaction with hydroperoxides, reaction (8), or by oxidation of the sulfide during autoxidation. Although active sulfoxides and thiolsulfinates are more effective inhibitors than the parent sulfides, their action is more short-lived (*15*). Therefore, it is generally advantageous to use sulfides which provide a reservoir from which active inhibitor can be generated in situ in low concentration. The instability of the sulfoxide compounds appears to be related to their inhibitory action (*16*). Thus, a very active sulfoxide, di-*t*-butyl sulfoxide, decomposes sr



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Kennerly and Patterson (*2*)
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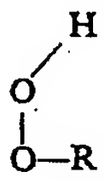

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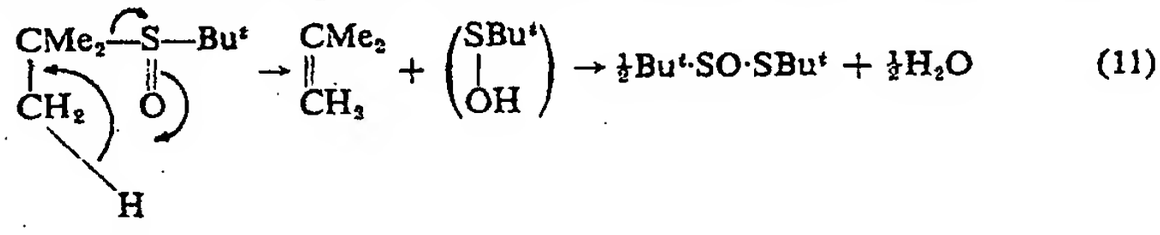
5). Thus, a very active sulfoxide,

di-*t*-butyl sulfoxide, decomposes smoothly in about 10 hr at 75°C (*17*):

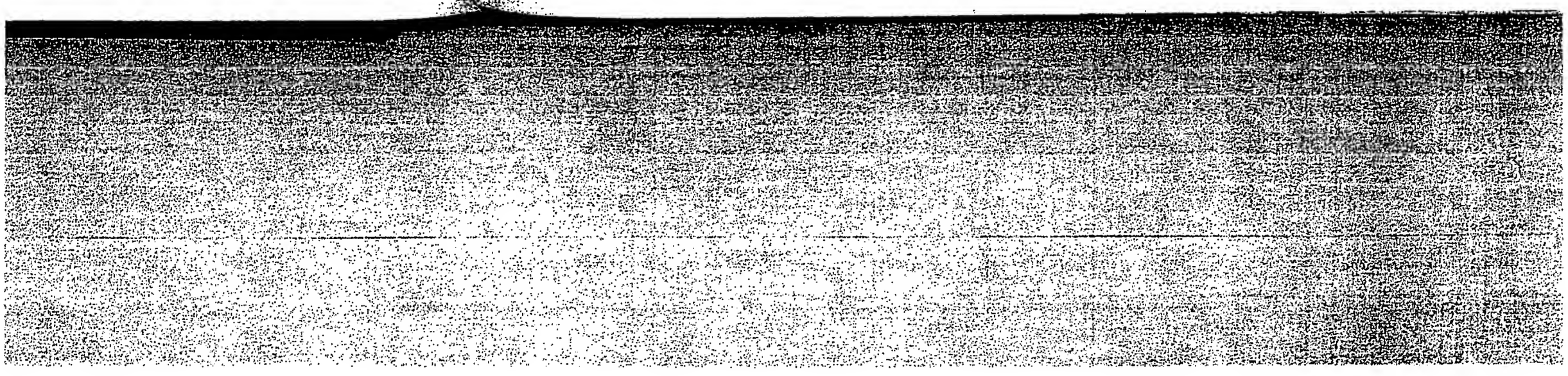


(11)

Evidence has also been accumulated to show that the active sulfoxide inhibitors do not interact with chain carriers in the propagation step. Thus, although they are active autoxidation inhibitors, they have little effect when the autoxidation is catalyzed by azoisobutyronitrile or by benzoyl peroxide. However, they remain effective when *t*-butyl hydroperoxide is added as an initiator (*16*). It is clear from the above evidence that sulfoxide compounds are active only when hydroperoxide decomposition is a necessary step in the autoxidation. The inhibitory process appears to involve initially a molecular association between hydroperoxide and sulfoxide. Such an association finds support in that the inhibition can be counteracted and suppressed by the addition of a bonding agent for the sulfoxide group, e.g., stearic acid. Additionally, it is necessary that the sulfoxide be present in equal or excess concentration to that of the hydroperoxide for the sulfoxide to exert a marked effect.

Kennerly and Patterson (*2*) studied the effect of a variety of sulfur-containing compounds as hydroperoxide decomposers for preoxidized mineral oil and for cumene hydroperoxide in mineral oil. They found that the rate of hydroperoxide disappearance was first order with respect to hydroperoxide concentration. This result might be anticipated for the preoxidized mineral oil which may contain products of peroxide decomposition, e.g., alcohols, where such products could function as hydrogen donors, reaction (10). However, it would not be expected for the cumene hydroperoxide [see reaction (9)]. Ingold (*18*), however, indicated that first-order hydroperoxide decomposition was plausible, providing a change in mechanism had occurred at the relatively higher temperature employed (150°C), where the hydroperoxide dimer concentration would be small [bimolecular kinetics, reaction (9), was observed at 50°C]. In view of reaction (8) it was further surprising when high yields of phenol, and not dimethylbenzyl alcohol, were reported for the cumene hydroperoxide decomposition in the presence of hydroxy sulfide derivatives. The high yield of phenol suggested the occurrence of an ionic rearrangement process of the type catalyzed by acids (sulfonic acids could be formed from the sulfur-containing additives):

$$RR'R''COOH + A \rightarrow RR'CO + R''OH + A \qquad (12)$$



where A = Lewis acid. When the R″ group is aryl, it will preferentially migrate to the exclusion of alkyl groups present. However, since no obvious process exists for the conversion of phenol sulfides to sulfonic acids, compatible with the observed dependence of activity upon structure, Kennerly and Patterson postulated that a mercaptyl radical or a phenoxy sulfide radical was the active species, and that sulfides were only precursors of the active species:

$$C_6H_5{-}C(CH_3)_2OOH + \cdot SR \rightarrow C_6H_5OC(CH_3)_2\overset{\oplus}{\dot{O}}H + \overset{\ominus}{S}R \longrightarrow C_6H_5O\cdot + CH_3COCH_3 + HSR \qquad (13)$$

$$C_6H_5O\cdot + HSR \rightarrow C_6H_5OH + \cdot SR \qquad (14)$$

$$C_6H_5OC(CH_3)_2\overset{\oplus}{\dot{O}}H + \overset{\ominus}{S}R \rightarrow C_6H_5OC(CH_3)_2SR + HO\cdot \text{ (termination)} \qquad (13a)$$

where ·SR = mercaptyl or phenoxy sulfide radical. Using concepts contained in reactions (13), (13a), and (14), the following scheme was postulated to account for the observed kinetic dependencies:

$$P \xrightarrow{k_{1a}} RO\cdot + HO\cdot \xrightarrow{2RH} ROH + H_2O + 2R\cdot \qquad (1')$$

$$RH + O_2 \xrightarrow{k_{1b}} R\cdot + HO_2\cdot \qquad (1b)$$

$$R\cdot + O_2 \xrightarrow{k_2} RO_2\cdot \qquad (2)$$

$$RO_2\cdot + RH \xrightarrow{k_3} P + R\cdot \qquad (3)$$

$$2RO_2\cdot \xrightarrow{k_6} \text{products} \qquad (6)$$

$$P + D \xrightarrow{k_{15}} Y + D' \qquad (15)$$

$$P + D' \xrightarrow{k_{16}} Z + D' \qquad (16)$$

$$P + D' \xrightarrow{k_{17}} \text{inert products} \qquad (17)$$

where P = hydroperoxide, D = original structure of peroxide decomposer, and D′ = active form of peroxide decomposer. We may write from the scheme, assuming steady state and high kinetic chain lengths,

$$k_{15}[P][D] = k_{17}[P][D'] \qquad (18)$$

$$k_{1a}[P] = k_6[RO_2\cdot]^2 \qquad (19)$$

$$k_{1a}[P] \gg k_{1b}[RH][O_2]$$

and from $d[P]/dt = 0$,

$$k_3[RH][RO_2\cdot] = k_{16}[P][D'] \qquad (20)$$

$$k_{16}[P][D'] \gg k_{1a}[P] + k_{15}[P][D]$$

Upon substituting Eqs. (18) anc
is obtained:

$$[P] = k_3^2 k_{1a}$$

From Eq. (21),

$$-\frac{d[D]}{dt} = k_{15}$$

Integrating Eq. (22) between th
[D] = 0, we obtain

t_{in}

where

$$k' = 2k_3^2$$

and t_{ind} = induction time.

Equation (23) has been verifie
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(Table 3-2).

2. *Phenols and Amines*

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phenols and hydroperoxides.

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$$_2\overset{\oplus}{O}H + \overset{\ominus}{S}R \longrightarrow$$
$$H_3 + HSR \longleftarrow \quad (13)$$
$$R \quad (14)$$

$_2SR + HO\cdot$ (termination) (13a)

radical. Using concepts
the following scheme was
dependencies:

$$.OH + H_3O + 2R\cdot \quad (1')$$
$$(1b)$$
$$(2)$$
$$(3)$$
$$(6)$$
$$(15)$$
$$(16)$$
$$(17)$$

ire of peroxide decomposer,
r. We may write from the
chain lengths,

$$] \quad (18)$$
$$(19)$$
$$O_2]$$
$$(20)$$
$$_{16}[P][D]$$

Upon substituting Eqs. (18) and (19) into Eq. (20), the following relation is obtained:

$$[P] = k_3^2 k_{1a} k_{17}^2 [RH]^2 / k_{16}^2 k_6 k_{15}^2 [D]^2 \quad (21)$$

From Eq. (21),

$$-\frac{d[D]}{dt} = k_{15}[P][D] = \frac{k_3^2 k_{1a} k_{17}^2 [RH]^2}{k_{16}^2 k_6 k_{15}[D]} \quad (22)$$

Integrating Eq. (22) between the limits, $t = 0$, $[D] = [D_0]$ and $t = t_{ind}$, $[D] = 0$, we obtain

$$t_{ind} = [D_0]^2 / k' \quad (23)$$

where

$$k' = 2k_3^2 k_{1a} k_{17}^2 [RH]^2 / k_6 k_{15} k_{16}^2$$

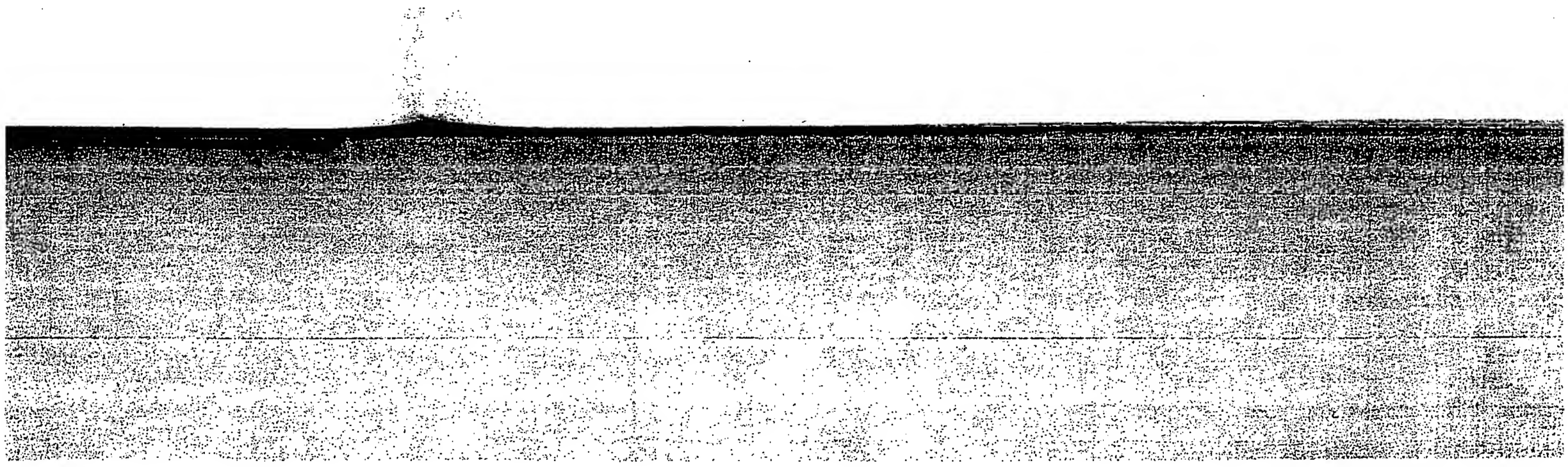
and t_{ind} = induction time.

Equation (23) has been verified experimentally for several hydroperoxide decomposers. Thus, when $\log(t_{ind})$ was plotted against $\log[D]$, straight lines were obtained each having a slope of 2 (Fig. 3-1). If steady-state peroxide concentration is quite low and initiation by direct attack of oxygen on substrate predominates, a similar derivation shows that the induction period, t_{ind}, is proportional to the first power of the initial decomposer concentration. In the presence of di-*n*-decyl sulfide (at certain concentrations), the induction time for mineral oil autoxidation was found to be proportional to the first power of the sulfide concentration.

It was surprising, as indicated earlier, that no dimethylbenzyl alcohol could be isolated by Kennerly and Patterson in their investigations, as would be expected from Eq. (8). In this connection it would be of interest to note the work of Oberright and co-workers (*19*) on the nature of the product resulting from cumene hydroperoxide (CHP) in the presence of sulfur-containing additives. They found that at an equimolar ratio of CHP to sulfur additive, the major product was dimethylbenzyl alcohol or derived products containing minor amounts of phenol. However, at very high molar ratios of CHP to sulfur additive (500:1) a catalytic decomposition occurred, yielding phenol as the only identified product (Table 3-2).

2. *Phenols and Amines*

There is relatively little information on the direct interaction between phenols and hydroperoxides. However, the interaction between phenols



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CHEMISTRY OF FREE RADICAL AND SINGLET OXIDATION OF LIPIDS

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CONTENTS

I. INTRODUCTION

The reaction of oxygen with unsaturated lipids produces a wide range of compounds that have attracted considerable interest and research. In foods, lipid oxidation products are responsible for the development of rancidity by the production of low molecular weight fission compounds that impart undesirable flavors. Potentially toxic and harmful cyclic and higher molecular weight materials are formed by thermal oxidation at the elevated temperatures of cooking and frying fats. In biological systems, enzymatic oxidation produces materials that are structurally related to those formed by non-enzymatic autoxidation. Much evidence points to the possible involvement of these lipid oxidation products in cancer, strokes, atheriosclerosis, inflammation, asthma, arthritis and the aging process. Many of these physiological effects of lipid oxidation have been well covered in other review articles.[27,49,77,105]

Another important process for the oxidation of unsaturated lipids involves activated species of oxygen. Singlet oxygen, produced by photo-oxidation in the presence of sensitizers such as chlorophyll, is an important reactant that has received much attention. Singlet oxidation provides a powerful tool in synthetic organic chemistry. The role that singlet oxygen and related species play in the hydroperoxidation of unsaturated lipids is now under intensive investigation.[28,49,71]

In the last decade much progress has been made in the chemistry of primary and secondary products of lipid oxidation, mainly as a result of advances in separation techniques and analytical methodology. This paper reviews structural studies of primary and secondary products of lipid oxidation. Free radical mechanisms for their formation are compared with that for singlet oxidation. Our current interests lie in further elucidating the structures of complex volatile and non-volatile decomposition products and in the mechanistic pathways for their formation. These decomposition products have a profound impact on the flavor and safety of lipid-containing foods, and they are implicated in many *in vivo* biological processes.[33,116]

II. HYDROPEROXIDATION

A. *Oleate*

Although the chemistry of autoxidation of unsaturated fatty acids has been under investigation for a long time,[8,25] only recently has sophisticated methodology been

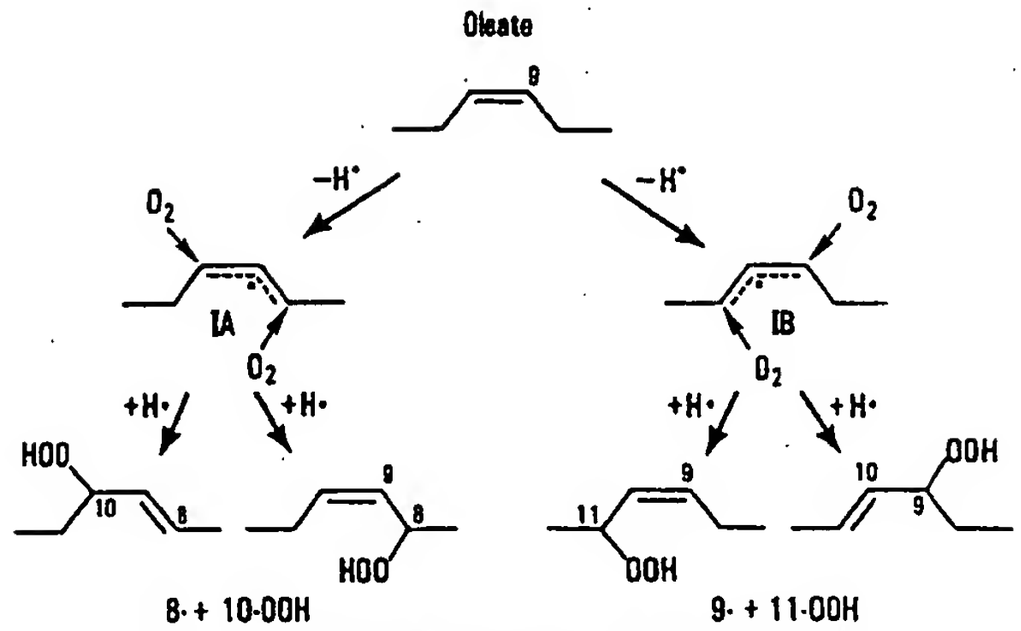


FIG. 1. Classical mechanism for oleate autoxidation.

available to determine the relative distribution of isomeric hydroperoxides and their stereochemistry, the effect of reaction conditions and the nature of secondary products.[30,31] The classical mechanism[29,30] for the free radical autoxidation of methyl oleate involves hydrogen abstraction at C-8 and C-11, producing two allylic radicals IA and IB (Fig. 1). Oxygen attack at the end carbon positions of the delocalized radicals IA and IB would produce four evenly distributed isomeric hydroperoxides: 9-hydroperoxy-*trans*-10-, 11-hydroperoxy-*cis*-9-, 10-hydroperoxy-*trans*-8- and 8-hydroperoxy-*cis*-9-octadecenoates. However, recent studies based on gas chromatography–mass spectrometry (GC–MS) and high performance liquid chromatography (HPLC) have shown that the 8- and 11-hydroperoxides are formed in larger proportions (26–28%) than the 9- and 10-hydroperoxides (22–24%).[41,82] Further ^{13}C-NMR studies showed the formation of *trans*-8- and *trans*-11-hydroperoxides as well as *cis*-9- and *cis*-10-hydroperoxides.[37,56] These results require a modification of the classical mechanism in Fig. 1.

A number of mechanisms to explain the formation of all eight *cis* and *trans* isomers of 8-, 9-, 10- and 11-hydroperoxides in autoxidized methyl oleate were previously discussed.[30] Mechanisms involving allylic isomerization via cyclic peroxide radical intermediates were postulated in the early literature[30] and supported recently by Piretti *et al.*[95] However, such cyclic intermediates are not consistent with the studies of Chan[15] on the isomerization of linoleate hydroperoxides, showing that the oxygen atoms in the hydroperoxy group are exchanged with atmospheric oxygen (cf Section II.B). In another mechanism, we invoked the loss of stereochemistry in the radical intermediates IA, IB, IIA and IIB (Fig. 2).[37] This isomerization of allylic radicals results in a change in conformation, especially at elevated temperatures, to form radical intermediates IIIA, IIIB, IVA and IVB. Temperature effects on the stereoisomeric composition of the allylic hydroperoxides reflect significant changes in the 8- and 11-hydroperoxides and very little in the 9- and 10-hydroperoxide isomers.[37,56]

In a recent study by Bascetta *et al.*,[6] ESR spectra taken during photolysis of di-*tert*-butyl peroxide solutions demonstrated the formation of allylic radicals regarded as IA and IB in methyl oleate and as IVA and IVB in methyl elaidate. Radicals IA and IB isomerized to IVA and IVB during photolysis, especially at elevated temperatures, and this change was shown to be irreversible. A mechanism was advanced in which the cisoid radicals IA and IB were interconverted either directly with the transoid radicals IVA and IVB (as shown in Fig. 2) or by reversible oxygen addition. However, the conditions of photolysis used by Bascetta *et al.* cannot be compared to those used in oleate autoxidation.[37] On one hand, methyl elaidate was found under their conditions of photolysis, whereas during free radical autoxidation only unchanged methyl oleate was observed. On the other hand, in contrast to autoxidation, which involves selective hydrogen abstraction by peroxyl radicals from allylic positions, hydrogen abstraction by *tert*-butoxyl radicals was shown to occur from non-allylic positions.

Under autoxidation conditions the small preference for the reaction of oxygen at C-8

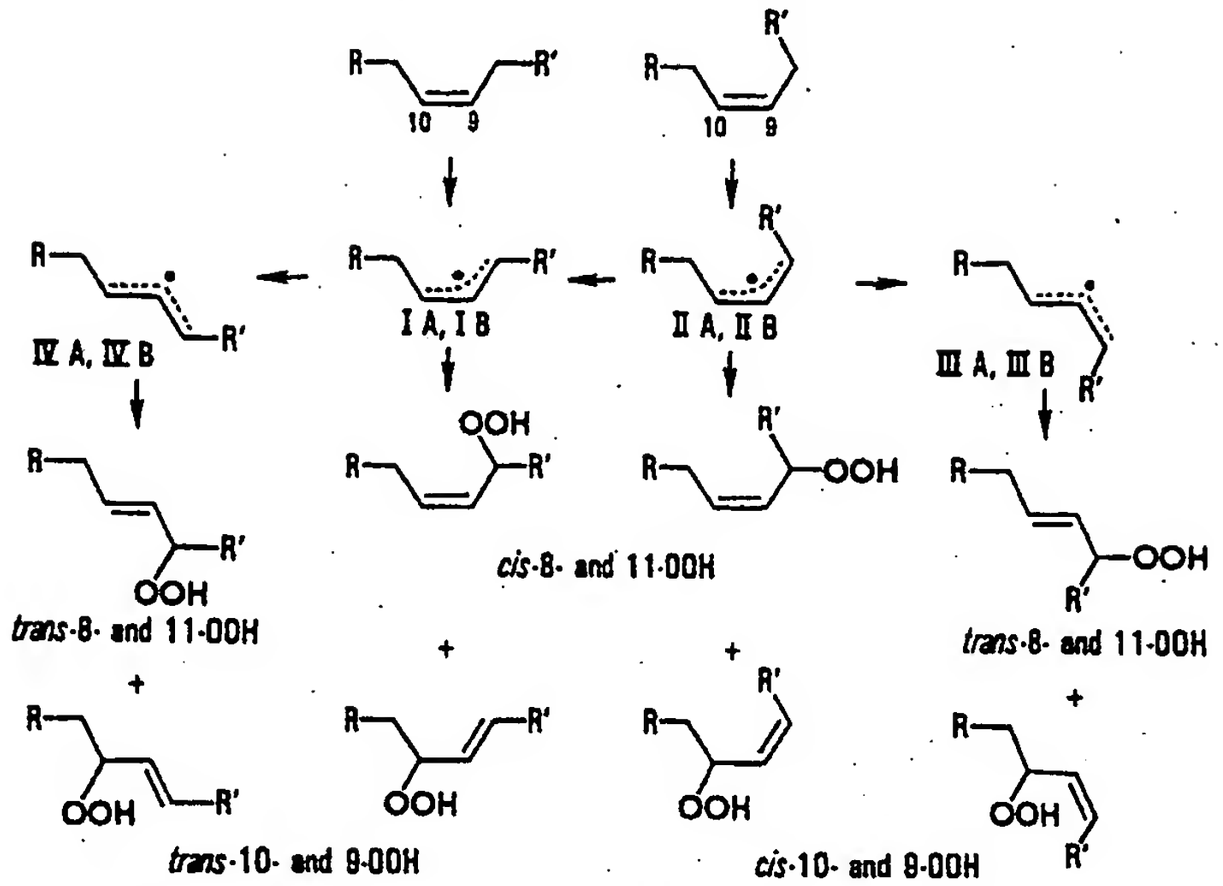


FIG. 2. Mechanism II for oleate autoxidation,[37] A, $R = CH_3(CH_2)_6$ and $R' = (CH_2)_6COOCH_3$, B, $R = (CH_2)_6COOCH_3$ and $R' = CH_3(CH_2)_6$.

and C-11 (about 4%), especially at low temperatures, may indicate some hydrogen abstraction from conformers of methyl oleate forming radicals that are not totally planar. Reaction with oxygen before rotation is complete would result in the observed small but mechanistically significant preference for the 8- and 11-hydroperoxides.

Singlet oxidation of methyl oleate takes an entirely different stereochemical course than free radical autoxidation. Singlet oxygen adds directly to either unsaturated carbon by an ene additon reaction,[23,28,50,59,69] and results in a change of configuration of the double bond from *cis* to *trans* (Fig. 3). By this mechanism only two products are formed in equal amounts, 9-hydroperoxy-*trans*-10- and 10-hydroperoxy-*trans*-8-octadecenoates.[11,20,40,82,117]

B. *Linoleate*

Early findings that free radical autoxidation of linoleate leads to the exclusive formation of an equal mixture of conjugated diene 9- and 13-hydroperoxides[24,29,117] were supported more recently by GC–MS studies showing equal amounts of these two hydroperoxide isomers at different levels of oxidation and wide range of temperatures.[42] These results support the classical mechanism[29,30] for linoleate autoxidation involving the formation of a pentadienyl hybrid radical V reacting preferentially with oxygen at the end 9- and 13-carbon positions (Fig. 4). The selective abstraction of hydrogen by peroxyl radicals VIA and VIB from C-11 of linoleate produces the corresponding 9-hydroperoxy-*trans*-10,*cis*-12- and 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoates.

Early infrared[10,104] and recent GC[42] and HPLC[13,99,100] studies showed that the hydroperoxides of methyl linoleate consist of a mixture of four *cis,trans* and *trans,trans*

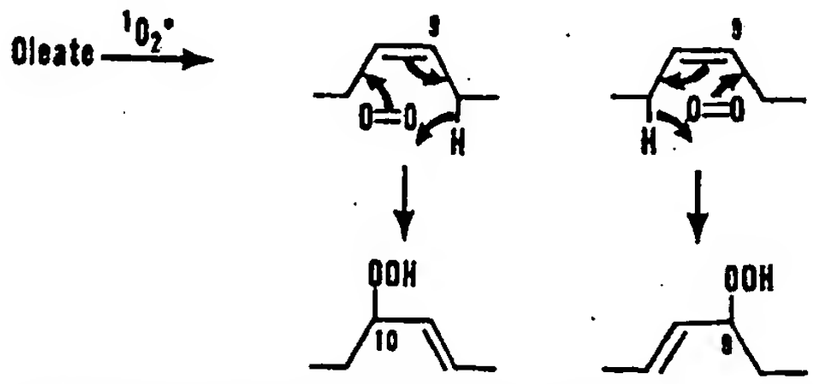


FIG. 3. Ene addition mechanism for singlet oxidation of methyl oleate.

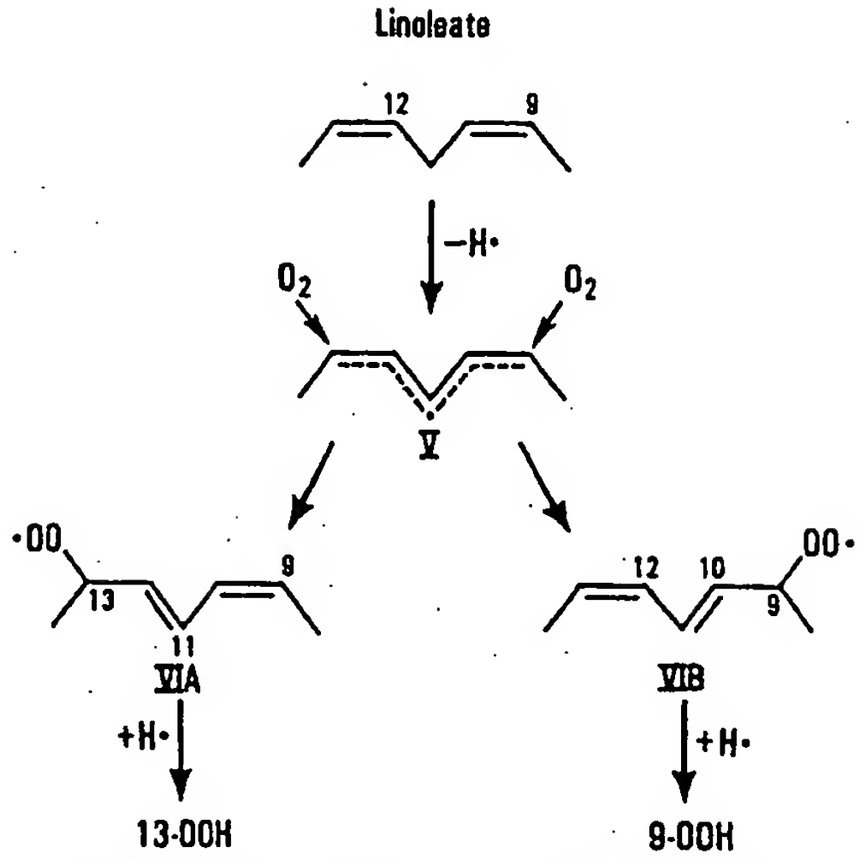
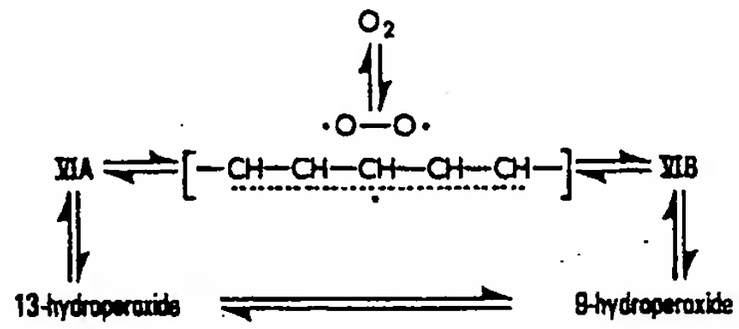


FIG. 4. Classical mechanism for linoleate autoxidation.

conjugated diene hydroperoxides and the relative proportion of the *trans,trans* isomers increases with the level and temperature of oxidation. By HPLC Chan *et al.*[12] showed that the *cis, trans*-9- and 13-linoleate hydroperoxides are readily interconverted when a hexane solution of each isomer was allowed to stand at 42°C for 15–40 hr. This thermal interconversion was accompanied by geometric isomerization to a mixture containing *trans,trans*-9- and 13-hydroperoxides. Using ^{18}O-labeled hydroperoxides, these workers later showed that the oxygen atoms of the OOH group exhanged with atmospheric oxygen during the thermal rearrangement.[15] They concluded on this basis that the reaction of oxygen with the pentadienyl radical of linoleate proceeds by a common pathway for both isomerization and autoxidation. The following mechanism, advanced by Chan *et al.*,



did not explain, however, the formation of all four *cis,trans*- and *trans,trans*-9- and 13-hydroperoxides of linoleate from either the 9- or 13-*cis,trans* isomer.

A mechanism explaining the stereochemistry of all four hydroperoxides formed from the autoxidation of linoleate was earlier postulated[30] in which the initial pentadienyl radical assumes four conformations before reaction with oxygen. Figure 5 shows a modification of this mechanism in which the initially formed *cis, cis*-pentadienyl radical V is converted to *trans,cis*/*cis,trans* radicals VII/VII′ and to *trans,trans* radical VIII at higher conversion and elevated temperatures. Under these conditions the ratio of *cis,trans* to *trans,trans* hydroperoxides decreases but the relative proportion of 9- and 13-hydroperoxides remains equal because all radicals V, VII and VIII react equally on C-9 and C-13 with oxygen. To explain the ready conversion of the *cis,trans*-9-hydroperoxide into a mixture of all four geometric isomers, observed by Chan *et al.*,[12,15] the reaction of the pentadienyl radicals V, VII and VIII with oxygen is assumed to be reversible. Upon loss of oxygen, the peroxy radicals (VIA,B, VIIA,B and VIIIA,B) revert into a pool of pentadienyl radicals (V, VII, VIII) that undergo rearrangement, as shown in Fig. 5.

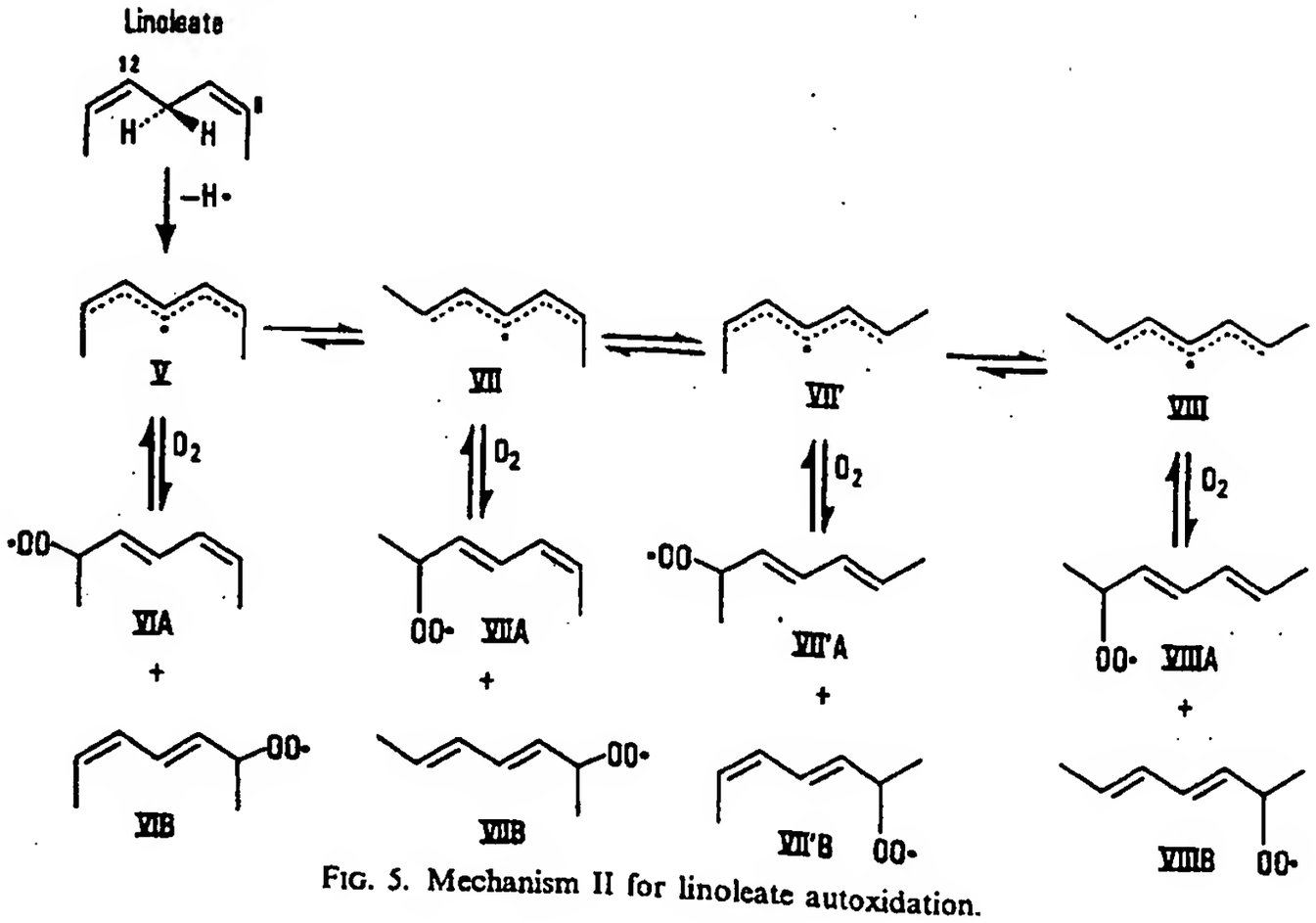
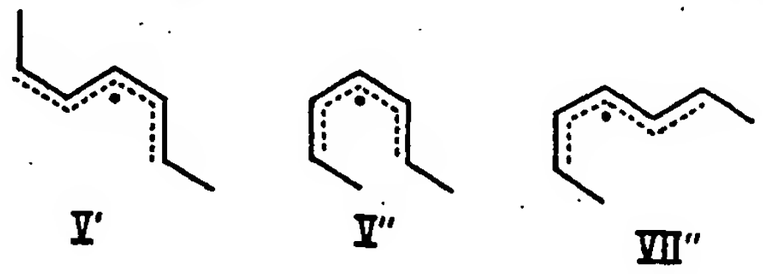
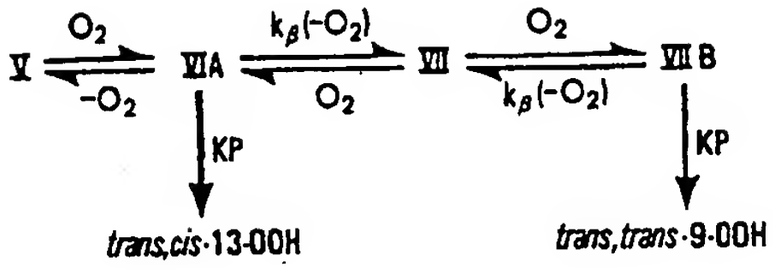


FIG. 5. Mechanism II for linoleate autoxidation.

The autoxidation of *cis,cis*-2,5-heptadiene, which is structurally equivalent to linoleic acid, produced equal proportions of *trans,cis*- and *trans,trans*-conjugated diene hydroperoxides at 25°C.[38] At 75°C the relative proportion of the *trans,trans* isomers increased to 90%. This significant geometric isomerization of the conjugated diene hydroperoxides was explained by a loss of stereochemistry of the pentadienyl radical by rotation about one of the carbon–carbon bonds to relieve the *cis* interactions between non-bonded atoms that strain a planar, completely delocalized radical. This change in stereochemistry of the pentadienyl radicals is implied in Fig. 5. Autoxidation of the other geometric isomers of 2,5-heptadiene produced mainly the *trans,trans* conjugated hydroperoxides (72–94%). The expected *trans*-3,*cis*-5 hydroperoxide was only produced from the *cis*-2,*trans*-5-heptadiene (10–22%) but not from the corresponding *trans*-2,*trans*,-5-diene. On the other hand, a significant amount of the *cis*-3,*trans*-5 hydroperoxide was produced from the *trans,trans* diene (6–10%) and less from the *cis,trans* diene (3–5%), which also produced the *cis,cis* hydroperoxide (to the extent of 3% at 25°C). These unusual hydroperoxide isomers were explained by the formation of pentadienyl isomers such as V′, V″ or VII″.



In another study by Porter *et al.*,[100] conditions were chosen in which the autoxidation of linoleic acid was kinetically controlled by using the free radical initiator di-*tert*-butyl hyponitrite. By limiting the HPLC analysis of isomeric hydroperoxides to less than 2% conversion, the *trans,cis*/*trans,trans* ratio of conjugated diene hydroperoxides was found to decrease with temperature (10–50°C) and to increase linearly with the concentration of the substrate. The stereochemistry of the ultimate hydroperoxides was proposed to be dependent on the configuration of the peroxyl radicals (VIA,B and VIIA,B) rather than that of the carbon pentadienyl radicals (V, VII) themselves (Fig. 5). According to this mechanism, the change in configuration from the *trans,cis* to the *trans,trans* hydroperoxides results from loss of oxygen, by β-scission, from the peroxyl radicals to give isomerized carbon radicals. The *trans,cis*/*trans,trans* product ratio was calculated by the relative competiton between β-scission of the carbon–oxygen bond of the peroxyl radicals



(k_β) and hydrogen abstraction of the same radicals (KP) leading to the corresponding hydroperoxides.

The addition of an antioxidant such as *p*-methoxyphenol, which acts as an excellent hydrogen donor, greatly increased the *trans,cis/trans,trans* ratio. The rate constant calculated for hydrogen transfer from this antioxidant was more than two orders of magnitude greater than the rate constant calculated for hydrogen transfer from linoleic acid.[100] In another study by the same group,[98] several hydrogen donors were tested as co-substrates with linoleic acid oxidized in benzene solution in the presence of di-*tert*-butyl peroxyoxalate as initiator. The *trans,cis/trans,trans* ratio was 27.3 in the presence of 1,4-cyclohexadiene, an excellent hydrogen donor, compared to 0.27 in the presence of cumene, a poor hydrogen donor. The addition of 5% α-tocopherol was shown to have the same effect as other hydrogen donors in completely inhibiting the formation of *trans,trans*-hydroperoxides during the autoxidation of methyl linoleate.[92]

With *cis,cis*-2,5-heptadiene, lower *trans,cis* to *trans,trans* ratios were observed[38] than those observed by Porter *et al.*[100] with linoleic acid, after 5 to 10% oxidation catalyzed with cobalt acetate. Therefore, at greater degrees of conversion, the stereochemistry of the allylic hydroperoxides may be influenced by thermodynamic as well as kinetic effects. Also, in neat lipid systems and in the presence of lipid oxidation radicals instead of *tert*-butoxyl radicals, different competing reactions may lead to different stereochemistry of allylic hydroperoxide products.

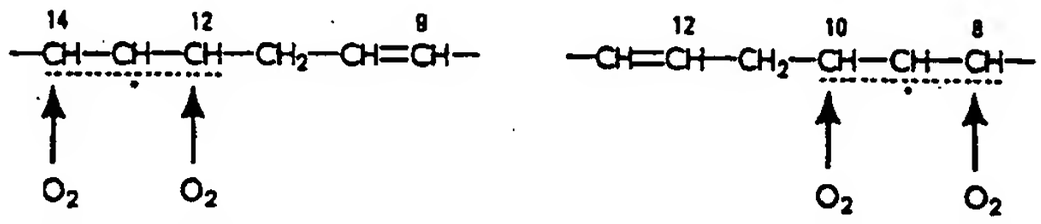
The results of Porter *et al.*[98,100] were confirmed by more detailed kinetic studies of the oxidation of methyl linoleate in acetonitrile solution by Yamamoto *et al.*[127] They reported that the ratio of *cis,trans* to *trans,trans* hydroperoxides was independent of oxygen pressure and dependent on solvent. This ratio increased with an increase in the dielectric constant of the solvent. These authors believe that solvents have greater effects on the propagation rate constant than on the rate constants for termination and β-scission of peroxy radicals.

ESR spectra taken during photolysis of di-*tert*-butyl peroxide solutions of methyl linoleate demonstrated the formation of the *cis,cis* radical V (Figs 4 and 5).[6] The corresponding *cis,trans* (VII) and *trans,trans* (VIII) radicals were observed from the photolysis of the isomeric *cis,trans* and *trans,trans* methyl linoleate esters. Under the conditions of these ESR experiments, no interconversion between V, VII and VIII was observed. These results were therefore considered by Bascetta *et al.*[6] to lend support to the mechanism of Porter *et al.*[100] that this interconversion proceeds via the peroxyl radicals VIA and VIB. It is not clear, however, why in the photolysis experiments with oleate[6] the *cis* radicals IA and IB are converted to the *trans* radicals IVA and IVB (Fig. 2) with the formation of methyl elaidate, whereas with linoleate the more favorable interconversion between *cis,cis* (V) and *cis-trans* radicals (VII) was contraindicated and no formation of *cis,trans* linoleate isomers was reported. Also with oleate, hydrogen abstraction by *tert* butoxyl radicals was shown to be non-selective and to occur from non-allylic positions, whereas neither secondary nor allylic radicals could be detected in the ESR spectra obtained during photolysis of methyl linoleate.

More recently, Porter and Wujek[102] showed that the autoxidation of the *trans,cis*, *cis,trans* and *trans,trans* isomers of methyl 9,12-octadecadienoate in chlorobenzene at 30°C produced the same distribution of *trans,cis* (0.05–0.11) and *trans,trans* (0.39–0.45) hydroperoxides as that from methyl linoleate. In the presence of increasing concentrations of cyclohexadiene as a hydrogen donor, the product hydroperoxides reflected the stereochemistry of the diene precursors. At 1.5–3 M concentrations of cyclohexadiene, these workers detected small amounts (less than 5 mol percent) of conjugated diene hydroperoxides other than the *trans,cis*- or *trans,trans*-9- and 13-hydroperoxides, but they were

not identified. Unusual *cis,trans* and *cis,cis* hydroperoxide isomers were also identified in an earlier study[38] with the geometric isomers of 2,5-heptadiene, which are structurally equivalent to those of methyl linoleate. When Porter and Wujek[102] reanalyzed their kinetic data with the geometric isomers of linoleate, they found significantly larger values for β-scission k_β of a *trans,cis* peroxyl radical, such as VIA (Fig. 5), than of a *trans,trans* peroxyl radical, such as VIIB. They generalized that the transoid end of the pentadienyl radicals has greater reactivity with oxygen than the cisoid end. A similar suggestion was made to explain the stereochemistry of hydroperoxides from *cis,trans*- and *trans,trans*-isomers of 2,5-heptadiene[38] by assuming that C-2 and C-6 in the pentadienyl radicals have different reactivities with oxygen.

In the presence of oxygen, with or without *tert*-butoxyl radicals, minor amounts of 8-, 10-, 12- and 14-hydroperoxides (4%) were recently reported by Grosch *et al.*[63,110] from the autoxidation of methyl and phenyl linoleate. These minor hydroperoxides are apparently derived by the non-selective hydrogen abstraction from the two non-allylic systems between C-8 and C-10 and between C-12 and C-14 in the linoleate radicals.



Singlet oxidation of methyl linoleate by the concerted ene addition mechanism (Fig. 3) produces a mixture of four isomeric hydroperoxides, two conjugated 9-hydroperoxy-*trans*-10-*cis*-12- and 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoates, and two unconjugated 10-hydroperoxy-*trans*-8-,*cis*-12- and 12-hydroperoxy-*cis*-9,*trans*-13-octadecadienoates.

Because linoleate reacts with singlet oxygen at a rate at least 1500 times faster than normal triplet oxygen, Rawls and van Santen[107] suggested that this very fast reaction is an important way of initiating the free radical autoxidation of unsaturated lipids. Hydroperoxides formed by singlet oxidation can decompose thermally or in the presence of metal catalysts into alkoxyl and peroxyl radicals that can accelerate free radical autoxidation. The decomposition of linoleic acid hydroperoxides catalyzed by metals, methemoglobin and hematin has also been shown to form singlet oxygen.[65] A free radical sensitized process can also occur by a type 1 photo-oxidation, in which the sensitizer reacts with the substrate.[28,50,59] Because both free radical autoxidation and singlet oxidation of methyl linoleate produce the same conjugated 9- and 13-hydroperoxides, the isomeric ratio of hydroperoxides can vary if these two processes occur concurrently. For all these reasons, it is very difficult to distinguish between singlet oxidation and free radical autoxidation.

Some reports[118,123] have indicated the formation of approximately equal amounts of the 9-, 10-, 12- and 13-hydroperoxides from the photosensitized oxidation of methyl linoleate, but other reports[19,40,119,120] have shown a product ratio favoring the conjugated 9- and 13-hydroperoxides. Terao and Matsushita[119,120] showed a change in isomeric ratio with level of oxidation. On the other hand, our quantitative studies[40,47,82] showed that the conjugated 9- and 13-hydroperoxides and the unconjugated 10- and 12-hydroperoxides were formed in a consistent ratio of about 2 to 1 over a wide range of peroxide values and reaction periods. By following the oxidation during successive dark and light periods, this isomeric distribution was shown to be due exclusively to photosensitized oxidation and thus characteristic of singlet oxygen.[47] The *cis* homoallylic structure of the internal 10- and 12-hydroperoxides of linoleate is a key feature leading to the formation of hydroperoxy epidioxides,[47,79] a subject that will be discussed in further detail in Section III.A. Therefore, the tendency of the peroxyl radicals from the internal 10- and 12-hydroperoxides to readily cyclize, accounts for their lower concentration than the external 9- and 13-hydroperoxide isomers.[47] Similarly, the analyses of minor hydroperoxides in autoxidized linoleate[63,110] showed larger concentrations of 8- and 14-isomers than 10- and 12-isomers, and hydroperoxy epidioxides derived from 10- and 12-hydroperoxides were identified by MS.

When singlet oxygen was produced chemically, using sodium hypochlorite and hydrogen peroxide, equal amounts of 9-, 10-, 12- and 13-hydroperoxide isomers from linoleate were reported by Terao and Matsushita.[120] However, these authors used butyl hydroxytoluene in the oxidation with chemically produced singlet oxygen, apparently to inhibit free radical autoxidation. It is now known that cyclization of the peroxyl radicals from homoallyic hydroperoxides of linolenate is effectively inhibited by hydrogen donors such as *p*-methoxyphenol[100] and α-tocopherol.[92] Therefore, the difference in distribution of isomers reported by Terao and Matsushita[120] between singlet oxygen produced chemically and by photosensitization is clearly not due to difference in mechanism of addition, but rather to the artifactual effect of the antioxidant used by them in inhibiting cyclization of the internal 10- and 12-hydroperoxyl radicals of linoleate.

C. *Linolenate*

The classical mechanism for the free radical autoxidation of methyl linolenate is based on that of linoleate (Fig. 4) and involves hydrogen abstraction on C-11 and C-14, producing two pentadienyl radicals IXA and IXB (Fig. 6). Oxygen attack at the end carbon positions of each pentadienyl radical produces a mixture of 9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15- and 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoates from radical IXA and 12-hydroperoxy-*cis*-9,*trans*-13,*cis*-15 and 16-hydroperoxy-*cis*-9,*cis*-12,*trans*-14-octadecatrienoates from radical IXB.

Our early study based on chemical degradation of the hydroxystearate derivatives[36] was fully confirmed later by GC–MS[43] and by HPLC[14,82] studies in showing a significantly higher proportion of outer 9- and 16-hydroperoxides (69–87%) than of internal 12- and 13-hydroperoxides (18–26%). We first suggested that the 12- and 13-hydroperoxides may be more easily decomposed.[36] Cyclization of these same hydroperoxide isomers was later

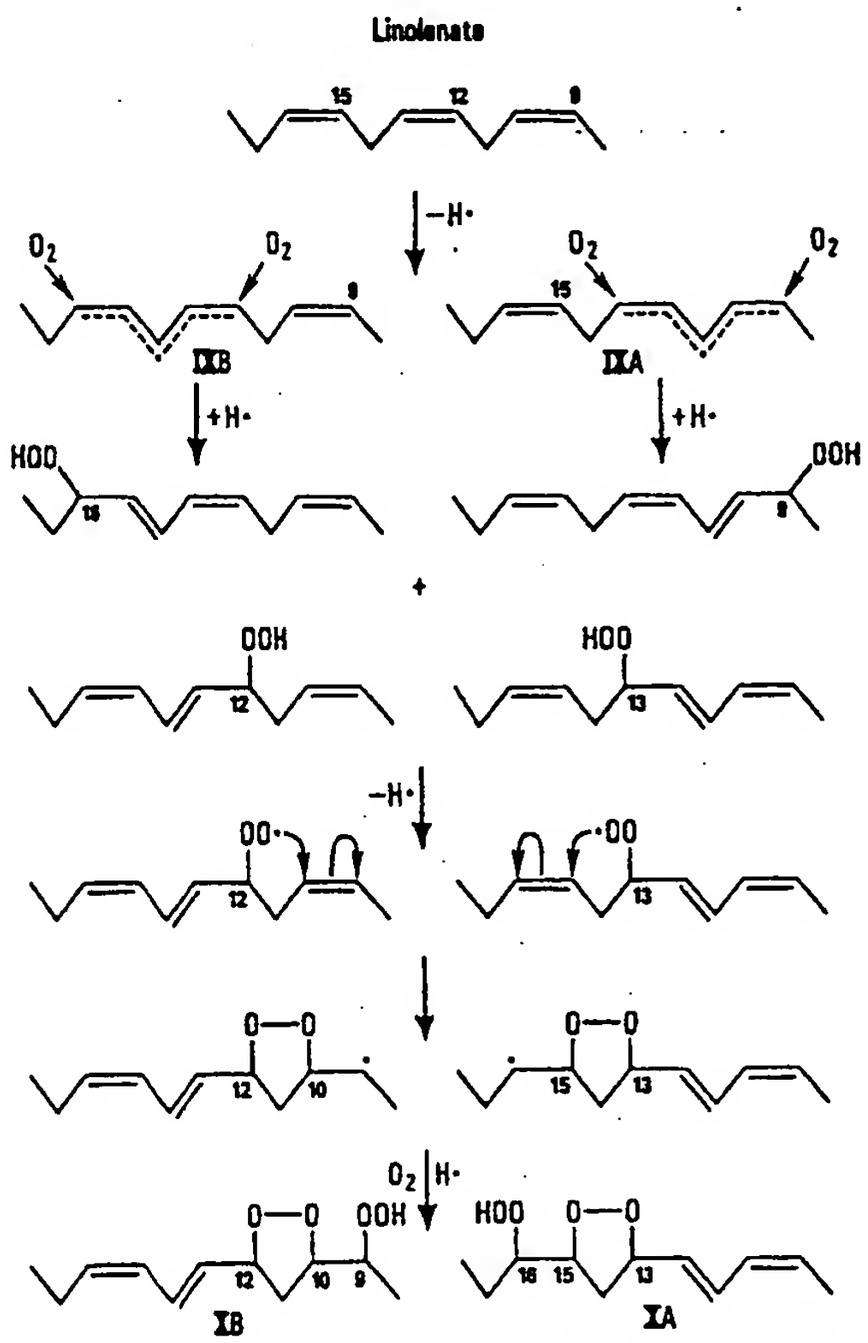


FIG. 6. Mechanism of autoxidation and oxidative cyclization of methyl linolenate.

indicated by indirect evidence for the formation of 9,10,12- and 13,15,16-trihydroxystearates by hydrogenation of the oxidation products of methyl linolenate.[43,64] Direct evidence was later established for the 1,3-cyclization of the 12- and 13-hydroperoxyl radicals (Fig. 6) by establishing the structures of the hydroperoxy cyclic peroxides (XA and XB) formed in autoxidized linolenate.[22,86,124] The *cis* homoallylic structure of the internal 12- and 13-hydroperoxides of linolenate permits the facile cyclization of their peroxyl radicals in the same way as indicated for the internal 10- and 12-hydroperoxyl radicals in singlet oxidized linoleate. The same uneven distribution of hydroperoxide isomers was found in autoxidized methyl linolenate at a wide range of conversion from 2 to 30% and temperatures from 25 to 80°C.[43] This finding indicates that cyclization of the 12- and 13-hydroperoxyl radicals is very rapid. Indeed, the yields of hydroperoxy cyclic peroxides (XA and XB) found in autoxidized methyl linolenate are of the same order of magnitude as the monohydroperoxides.[86]

The 16-hydroperoxide isomer is also formed at a higher concentration (43–52%) than that of the 9-hydroperoxide isomer (27–35%) in autoxidized methyl linolenate.[36,43] Therefore, oxygen attack may be preferred on C-16 closest to the end of the fatty acid chain of linolenate. This selective oxygen attack at the end of the fatty molecule is further supported by our studies of the autoxidation of methyl 9,15- and 12,15-octadecadienoates.[34] According to the classical mechanisms for oleate autoxidation (Fig. 1), methyl 9,15-diene would produce the same allylic 8-, 9-, 10- and 11-hydroperoxide isomers as methyl oleate by reaction of oxygen with the Δ9 double bond. On the other hand, autoxidation of the Δ15 double bond would produce a mixture of allylic 14-, 15-, 16- and 17-hydroperoxides. Our GC–MS analyses showed that the proportion of 16- (15%) and 17-hydroperoxides (22%) was significantly higher than that of the other isomers (8–12% each: 8-, 9-, 10-, 11-, 14- and 15-hydroperoxides). In the same way, the normalized concentration of 16-hydroperoxide from 12,15-octadecadienoate was larger (58%) than the 12-hydroperoxide (42%). Therefore, both isomeric dienes produce allylic radicals in which the carbons closest to the end of the fatty acid chain (C-16 and C-17) are more reactive with oxygen. These results show that with linolenate the uneven distribution of hydroperoxide isomers is not only determined by the tendency of the inner 12- and 13-hydroperoxy radicals to rapidly cyclize, but also to the greater reactivity with oxygen of C-16 of radical IXB closest to the end of the fatty acid chain. The addition of 5% α-tocopherol as a hydrogen donor was shown to inhibit the cyclization of the internal 12- and 13-hydroperoxyl radicals of linolenate and to produce an even distribution of the 9-, 12-, 13- and 16-hydroperoxide isomers.[92]

Singlet oxidation of methyl linolenate by the concerted ene addition mechanism (Fig. 3) produces the expected mixture of six hydroperoxides with OOH substituted on each unsaturated carbon 9, 10, 12, 13, 15 and 16.[11,20,40,82,87,120] As with linoleate, we found that the outer 9- and 16-hydroperoxide isomers are formed in significantly higher proportion (20–29%) than the inner 10-, 12-, 13- and 15-hydroperoxide isomers (12–16%).[40,82,87] As discussed below, the inner hydroperoxide isomers have a homoallylic structure leading to cyclization of their peroxyl radicals (Section III.A).

D. *Arachidonate*

By the same mechanisms for free radical autoxidation of methyl linoleate (Fig. 4) and linolenate (Fig. 6), arachidonate forms three pentadienyl radicals XIA, XIB and XIC by hydrogen abstraction on, respectively, C-7, C-10 and C-13 (Fig. 7). Oxygen addition at either end of the pentadienyl radicals produces a mixture of six isomers with hydroperoxide substitution on carbons 5, 8, 9, 11, 12 and 15.

Porter *et al.*[101] separated by HPLC the *cis,trans*- and *trans,trans*-conjugated diene hydroxy derivatives from autoxidized methyl arachidonate, and the corresponding *cis,trans* compounds from autoxidized arachidonic acid. Although the HPLC chromatograms indicated that the external 5- and 15-hydroperoxide isomers were the dominant products, no quantitative isomeric composition was reported. By GC–MS Matsushita *et*

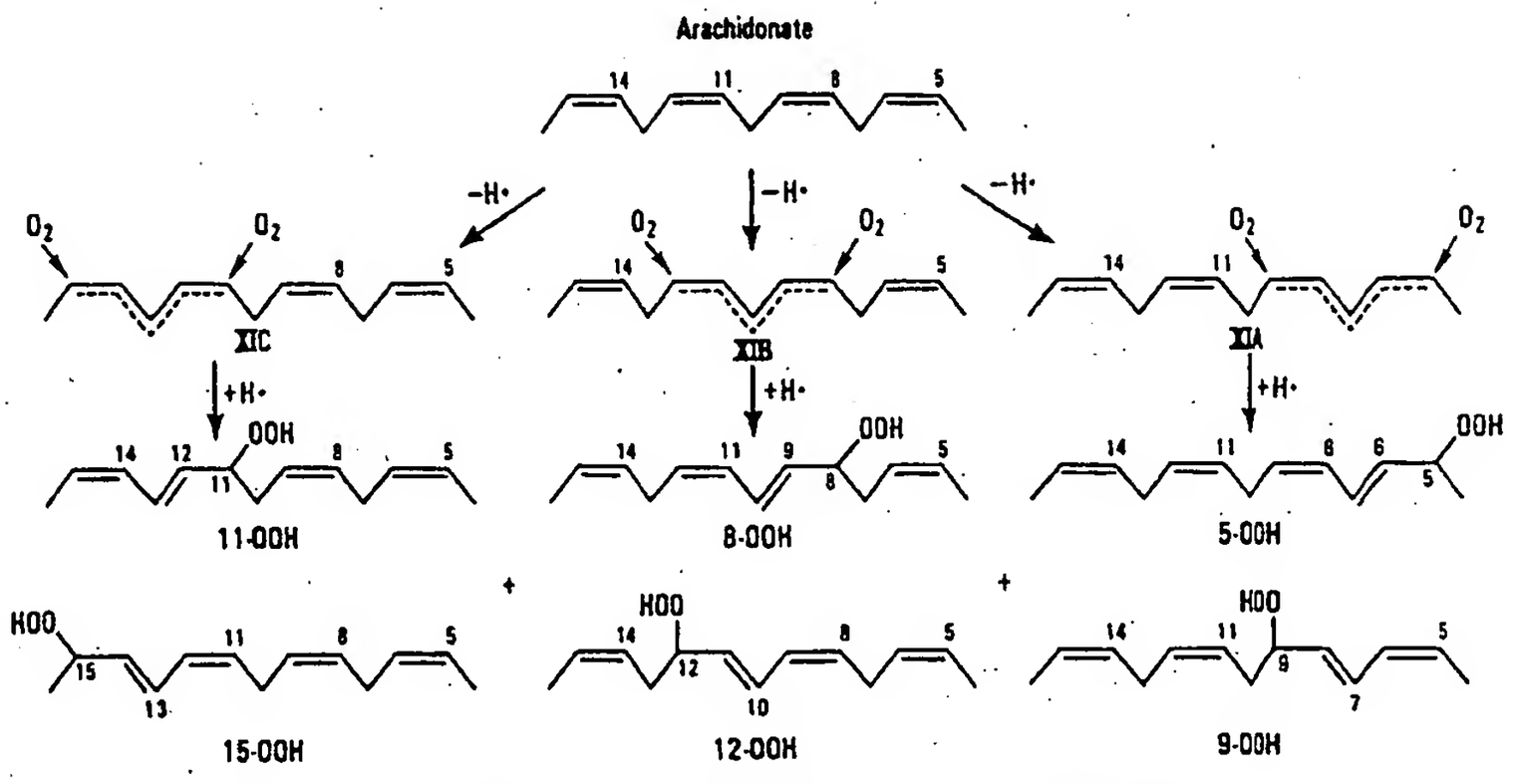


FIG. 7. Mechanism of autoxidation of methyl arachidonate.

al.[122,125] found significantly higher concentrations of the outer 5-hydroxy (18.3%) and 15-hydroxy (33.8%) than of the inner 8-, 9-, 11- and 12-hydroxy (9.8–15.6%) isomers in methyl arachidonate autoxidized at 35°C. Oxidation of either arachidonic acid or its methyl ester in an emulsion system catalyzed by hemoprotein produced approximately equal amounts of 5- and 15-hydroxy (24–31%), of 9- and 11-hydroxy (12–16%), and of 8- and 12-hydroxy (5–10%) isomers.[124] The distribution of hydroperoxide isomers in autoxidized arachidonate is of the same type as that found in autoxidized methyl linolenate,[14,36,43,82] and can be similarly attributed to the tendency for the inner hydroperoxide isomers (8-, 9-, 11- and 12-OOH) to cyclize.

Porter *et al.*[98] oxidized arachidonic acid in mixtures of benzene/1,4-cyclohexadiene as hydrogen donor, with linoleic acid as internal standard. They devised a kinetic expression to estimate the rate of cyclization of the internal peroxyl free radicals formed from arachidonic acid and assumed that oxygen addition is comparable at either end of the pentadienyl radicals XIA, XIB and XIC (Fig. 7). However, the results of Terao and Matsushita[122,125] show that oxygen is more reactive at the end C-15 position than at the C-11 position of radical XIC. Arachidonate appears to behave the same way as linolenate on autoxidation, producing an uneven distribution of isomeric hydroperoxides determined both by the cyclization of the internal 8-, 9-, 11- and 12-peroxyl radical isomers and by the greater reactivity with oxygen of C-15 of radical XIC closest to the end of the fatty acid chain. As with methyl linolenate,[92] the addition of 1–5% tocopherol to arachidonate resulted in an even distribution of the six hydroperoxide isomers due to inhibition of cyclization of the internal 8-, 9-, 11- and 12-hydroperoxyl radical isomers.[91,124]

The oxidation of arachidonic acid and methyl arachidonate with singlet oxygen produces a mixture of eight hydroperoxide isomers (substituted on carbon 5, 6, 8, 9, 11, 12, 14 and 15) as expected by the concerted ene addition mechanism (Fig. 3).[99,121,122] As with linoleate and linolenate, Terao and Matsushita[121,122] found that the outer 5- and 15-hydroperoxide isomers are formed in much higher concentrations (16–25%) than the inner 6-, 8-, 9-, 11-, 12- and 14-isomers (4–13%). Products from cyclization of the peroxyl radicals derived from these internal hydroperoxide isomers include monocyclic and bicyclic peroxides as well as epoxy alcohols[99] (Section III.A).

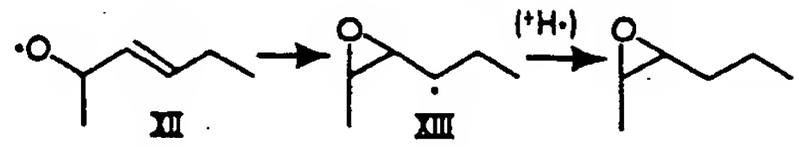
III. SECONDARY OXIDATION

A. *Monomeric Products*

On prolonged oxidation, monohydroperoxides are converted into oxygenated side products of the same chain length. A multitude of secondary oxidation products were

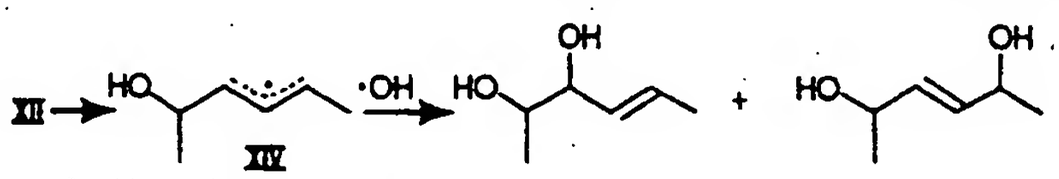
identified by GC–MS analyses of oxidized methyl oleate, linoleate and linolenate after derivatization. From autoxidized methyl oleate[41] small amounts of allylic ketones (with CO on carbon-8, -9, -10 and -11), saturated epoxy esters (8,9-, 9,10- and 10,11-), dihydroxyenes (8,9-, 9,10-, and 10,11-) and saturated dihydroxy esters (with one hydroxy scattered between C-8 and C-10 and the other between C-9 and C-11) are found. These secondary products were also separated by HPLC after reduction and identified as isomeric mixtures of dihydroxy esters, hydroxyenes (derived from corresponding hydroperoxides) and epoxystearate.[84] Similarly, methyl 9,10-epoxystearate (threo and erythro) was identified in autoxidation mixtures of methyl oleate,[72,78] as well as isomeric mixtures of allylic keto esters (8-, 9-, 10- and 11-isomers), the corresponding hydroxy esters and their epoxy derivatives.[72]

The 9,10-epoxystearate is apparently derived mainly by peroxide addition to the double bond of methyl oleate, whereas the other positional isomers may come from rearrangement of oxy radicals from the corresponding hydroperoxides. For example, the following cyclization of an alkoxyl radical leads to the 10,11-epoxy ester from the 11-hydroperoxide,



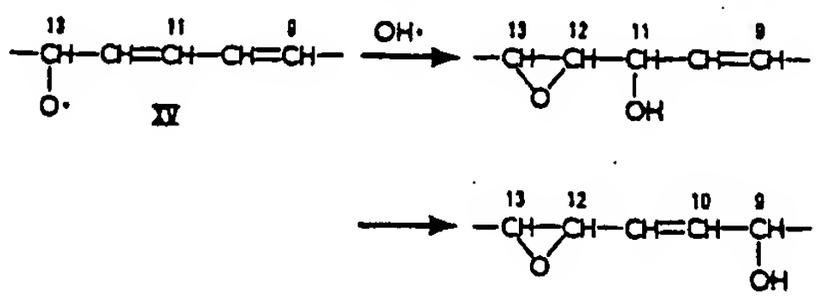
the 8,9-epoxy ester from the 8-hydroperoxide, and the 9,10-epoxy ester from both 9- and 10-hydroperoxides.[84] The allylic ketones come from the alkoxy radical XII by oxidative removal of H· by another species, and the corresponding hydroxy epoxy derivatives by addition of ·OH to radical XII.

The dihydroxy esters from oxidized methyl oleate were postulated to come from radical XII by OH and H radical substitution or via the formation of hydroxy radical XIV.[32]



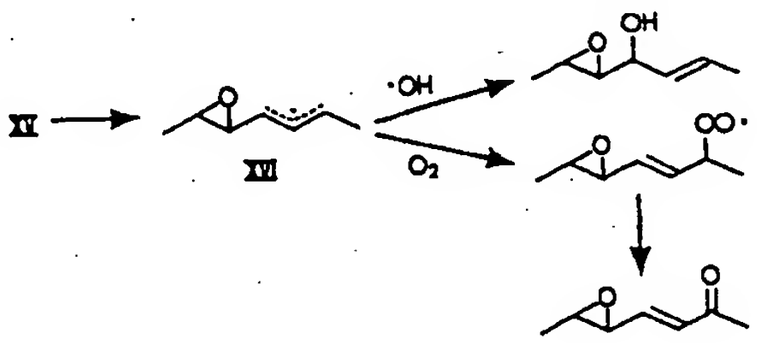
From autoxidized methyl linoleate, keto dienes (with CO on C-9 and C-13), epoxy hydroxyenes (9,10-/12,13-epoxy-11-hydroxy and 9/13-hydroxy-12,13/9,10-epoxy), dihydroxy (9,13-) and trihydroxy (9,10,13- and 9,12,13-) esters were found.[42,84] Terao and Matsushita[117] allowed methyl linoleate hydroperoxides to decompose at 37°C for a week and identified among the secondary products (after chemical reduction) 9,13-dihydroxy and trihydroxy (9,12,13- or 9,10,13-) esters.

Significant progress in identifying the secondary oxidation products of linoleate has been possible by the availability of pure 9- and 13-hydroperoxide isomers through the action of specific lipoxygenases. Hamberg and Gotthammar[62] identified threo-11-hydroxy-12,13-epoxy-9-octadecenoic acid as a major product from the decomposition of *cis,trans*-13-hydroperoxide of linoleic acid at 100°C in aqueous ethanol. The same hydroxy epoxy isomer, together with the 11-hydroxy-9,10-epoxyene, was also identified from autoxidized linoleic acid containing both 13- and 9-hydroperoxide isomers. Hamberg[61] later decomposed the 13-hydroperoxide isomer of linoleic acid in the presence of hemoglobin, and identified the corresponding 13-keto and hydroxy dienes together with the erythro- and threo-11-hydroxy-12,13-epoxy-9-monoene isomers and 9-hydroxy-12,13-epoxy-10-monoene. The mechanism suggested for the formation of hydroxy epoxy compounds involves cyclization of an oxy radical intermediate XV, followed by hydroxy radical addition with and without double bond migration. The same mechanism was suggested

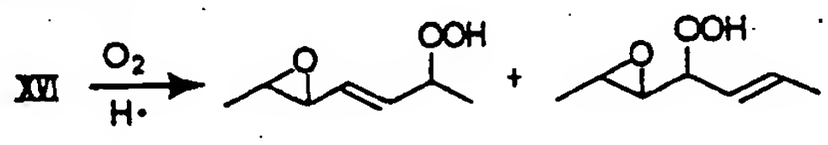


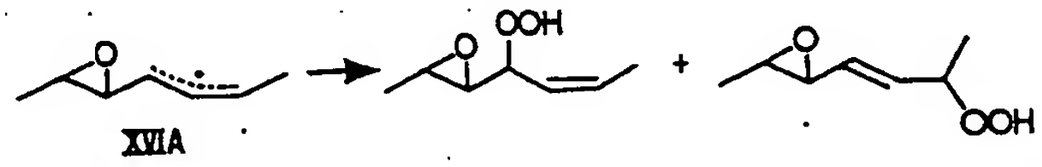
earlier by Maier and Tappel[74] to explain the formation of hydroxy epoxide from the decomposition of linoleate hydroperoxides with hematin compounds.

Gardner *et al.*[54] studied the homolytic decomposition of linoleic hydroperoxides catalyzed by an Fe(III)–cysteine system in ethanol–water solution, and identified isomeric mixtures of keto dienes, hydroxy dienes, hydroxy epoxy and keto epoxy monoene compounds, together with ethoxy esters apparently derived by solvolysis. For the epoxy compound, they suggested either a free radical or an ionic mechanism for cyclization of the hydroperoxy group to the α-unsaturation. Gardner[52] later detailed a free radical mechanism involving an allylic epoxy radical XVI, reacting either with ·OH in the



α-position or with O_2 in the γ-position leading to the corresponding epoxy keto compound. This mechanism was most recently refined by assuming that the allylic epoxy radical has either the *cis* conformation XVIA or *trans* conformation XVI to account for the formation of *trans*-12,13-epoxy-9-oxo-*trans*-10-monoene, *cis*-12,13-epoxy-9-oxo-*trans*-10-monoene, the corresponding 9-hydroxy compound, and the 11-hydroxy-*cis*/*trans*-9-monoene (erythro/threo) compounds.[53]





Decomposition of the 13-hydroperoxide of methyl linoleate in the presence of di-*tert*-butyl peroxyoxalate at 38°C produced a mixture of 9- and 13-keto dienes, keto-epoxy monoenes, hydroxy-epoxy monoenes, keto-dihydroxy and trihydroxy esters.[112] The mechanism suggested includes positional isomerization of peroxy radicals followed by disproportionation into a mixture of keto dienes and hydroxy dienes, which react with peroxide oxygen across a double bond. In an alternate pathway suggested, a caged alkoxy radical cyclizes to the α-unsaturation and the resulting epoxy radical is peroxidized. Photolysis of the 13-hydroperoxide of methyl linoleate in methanol under anaerobic conditions produced epoxy-hydroxymethyl-, epoxy-hydroxy- and hydroxy-methoxy-esters as major products.[111] The mechanism suggested involves photolysis of the hydroperoxide with the formation of OH radicals that react with the methanol solvent to produce CH_2OH and OCH_3 radicals reacting with epoxy allylic radicals such as XVI and XVIA.

The formation of di- and trihydroxy esters from oxidized methyl linoleate[42,84,117] can also be explained by a mechanism involving either the allylic epoxy radical XVI by hydration or the dienoic alkoxyl radical XV undergoing 1- or 3-addition of OH.[32] Other possible precursors of these polyoxygenated products include dihydroperoxides, keto-hydroperoxides and hydroxy-hydroperoxides.[32,84]

Secondary oxidation products reported from methyl linoleate treated with singlet oxygen include epoxy esters,[47] di- and trioxygenated compounds,[120] hydroperoxy cyclic peroxides,[47,79] an unsaturated epoxy alcohol[79] and dihydroperoxides.[47] By separating four isomeric hydroperoxy cyclic peroxides in sensitized photo-oxidized methyl linoleate (Fig. 8), we were able to prove that they come from the internal 10- and 12-hydroperoxides.[47] These isomers have the same homoallylic unsaturation as the internal 12- and

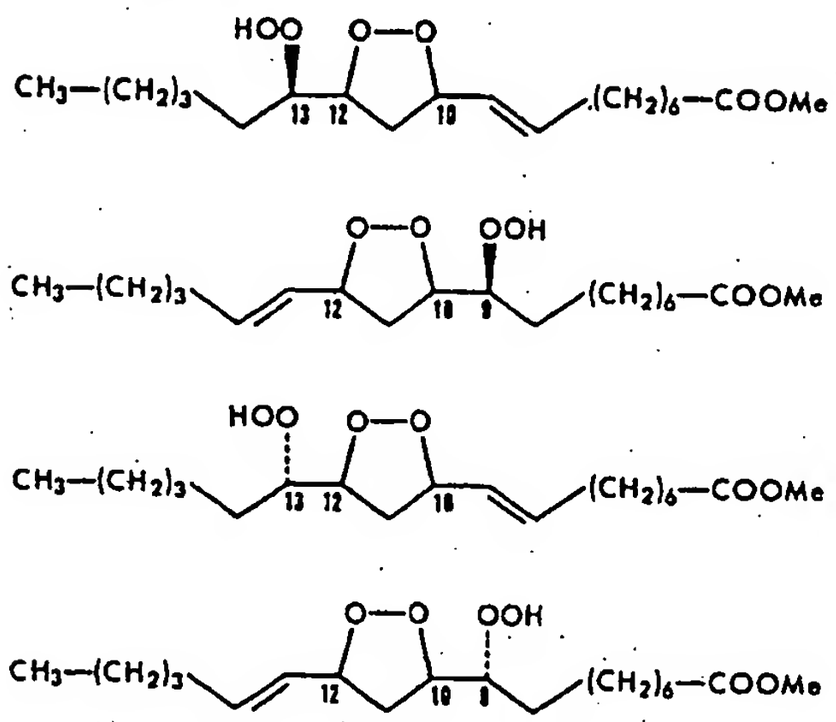


FIG. 8. Structures of hydroperoxy epidioxides from methyl linoleate treated with singlet oxygen.[47]

13-hydroperoxides of linolenate, which is required for cyclization (Fig. 6). Cyclization of the 10- and 12-hydroperoxyl radicals of linoleate accounts for their lower relative concentrations (31–42%) than the conjugated 9- and 13-hydroperoxides (58–69%) observed at a wide range of oxidation levels[47] (Section II.B). We also observed that the hydroperoxy cyclic peroxides of methyl linoleate (Fig. 8) are formed readily by allowing the pure monohydroperoxides of singlet oxidized linoleate to stand in the dark at 0–5°C. This evidence indicates that cyclization is a rapid free radical process occurring as a side reaction and not photosensitized. The dihydroperoxides identified in singlet oxidized linoleate were isomeric mixtures with one hydroperoxide group between C-9 and C-12 and the other hydroperoxide between C-10 and C-13.[47]

The photosensitized oxidation of a mixture of 9- and 13-hydroperoxides from methyl linoleate produced six-membered cyclic peroxides by 1,4-addition of singlet oxygen to the conjugated diene systems.[85] Because little change in the relative amount of *trans,trans* conjugated diene was observed during this reaction, a mechanism was postulated in which photosensitized isomerization from *cis,trans* to *trans,trans*[19] occurs at a much faster rate (k_1/k_2) than 1,4-cycloaddition (k_3) of singlet oxygen to the conjugated diene system of linoleate hydroperoxides (Fig. 9).

From autoxidized methyl linolenate, we identified by GC–MS of the hydrogenated derivatives, significant amounts of 9,10,12- and 13,15,16-trihydroxy esters that were taken as indirect evidence for hydroperoxy cyclic peroxides XA and XB (Fig. 6).[43] The same evidence for either six- or five-membered hydroperoxy cyclic peroxides was reported earlier by Haverkamp Begeman *et al.*[64] Pryor *et al.*[106] also reduced the thermal decomposition products of autoxidized methyl linolenate to obtain indirect evidence for hydroperoxy bicyclic endoperoxides by GC–MS based on trihydroxy unsaturated cyclic compounds.

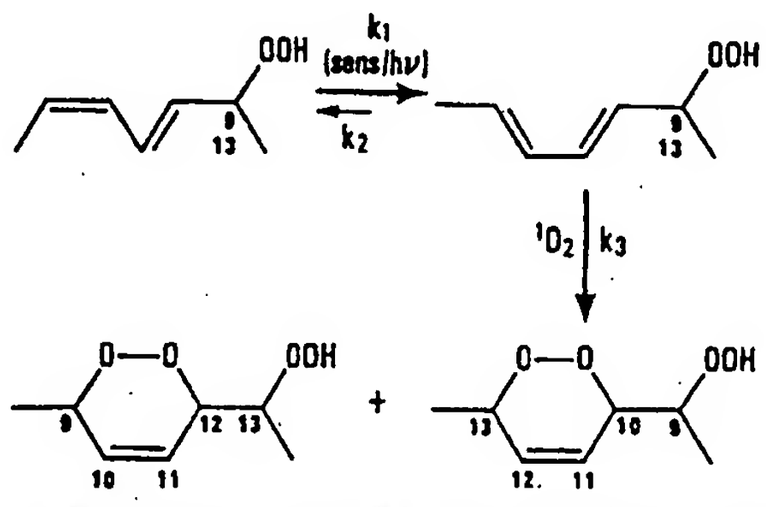
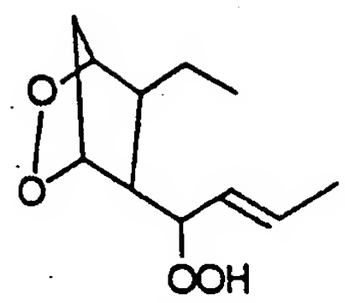
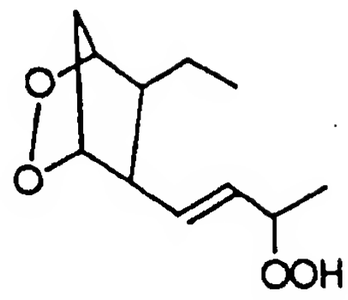


FIG. 9. Scheme for the formation of six-membered hydroperoxy epidioxides from linoleate hydroperoxides (sens = sensitizer).[85]



XVIIA



XVIIB

They postulated a mechanism in which the cyclic radical precursors of XA and XB (Fig. 6) would form isomeric bicycloendoperoxides, with allylic hydroperoxide group substituted either in the α (XVIIA) or γ (XVIIB) position relative to the ring structure. These bicyclic endoperoxides, formed from polyunsaturated fatty acids containing three or more double bonds, were suggested as important precursors of malonaldehyde formed by acid catalyzed or thermal decomposition and as the source of the thiobarbituric acid (TBA)-reactive materials in autoxidation systems. In earlier studies on the enzymatic conversion of all *cis*-8,11,14-eicosatrienoic acid into prostaglandin E_1, Nugteren *et al.*[88] observed the formation of large amounts of 12-hydroxyheptadecadienoate together with equimolar amounts of malonaldehyde as measured by the TBA reaction. They also suggested that an endoperoxide intermediate may be an important source of malonaldehyde.

From autoxidized methyl linolenate, we identified hydroperoxy cyclic peroxides XA and XB (Fig. 6) as mixtures of *cis,trans* and *trans,trans* diene isomers, in yields similar to the monohydroperoxide precursors.[86] From the structures of these monocyclic compounds, we concluded that they are derived from the cyclization of the 12- and 13-hydroperoxyl radicals of linolenate, which accounts for the significantly lower concentration of these hydroperoxide isomers relative to the 9- and 16-isomers (Section II.C). Dihydroperoxides were identified in smaller yields as a mixture of 9,12-, 9,16- and 13,16-isomers. These dihydroperoxides are those expected from the secondary oxidation via pentadienyl radical intermediates formed by hydrogen abstraction on C-11 of the 16-hydroperoxide and on C-14 of the 9-hydroperoxide of methyl linolenate (Fig. 6), as predicted previously.[29] Epoxy-hydroxy or epoxy-hydroperoxy dienes were also identified in minor amounts in autoxidized methyl linolenate.[86]

Coxon *et al.*[22] independently identified the same *cis,trans* and *trans,trans* isomeric hydroperoxy cyclic peroxides XA and XB (Fig. 6), either by autoxidation of pure *cis,trans*-dienoic 9- and 13-hydroperoxides of methyl linolenate (prepared enzymatically) or from autoxidized methyl linolenate. Autoxidation of pure *cis,trans*-13-linolenate hydroperoxide led to the formation of the expected 16-hydroperoxy epidioxide XA in a mixture of three stereoisomers (7.5%) accompanied by 1% 9-hydroperoxy epidioxide XB. They presumed, therefore, that the precursor pentadienyl radicals IXA and IXB (Fig. 6) were interconverted. Autoxidation of pure *cis,trans*-9-linolenate hydroperoxide led to the formation of a mixture of 16-hydroperoxy epidioxide stereoisomers of XA (16%) and *cis,trans* 9-hydroperoxy epidioxide XB (7%). This result was also explained by rearrangement of the pentadienyl radicals IXA and IXB, leading to the 12- and 13-hydroperoxy radicals, which produce cyclic peroxide XB and XA, respectively (Fig. 6).

On the basis of the work of Porter *et al.*,[98,100] these rearrangements of the 9- and 13-hydroperoxide isomers would not be expected during autoxidation of linolenate, not only because cyclization of the 12- and 13-hydroperoxyl radicals is dominant, but also because the presence of large amounts of unreacted linolenate would prevent isomerization and rearrangements. These isomerization processes, therefore, become significant only when cyclization of pure hydroperoxides is carried out *in the absence* of unreacted linolenate. Indeed, hydroperoxy cyclic peroxides XA and XB have been identified as significant products when the oxidation of methyl linolenate was catalyzed with Fe(II)–ascorbic acid but not when the pure monohydroperoxides of linolenate were oxidized under the same conditions.[124] Therefore, it is important to distinguish between oxidation experiments in which the substrate linolenate is present in large excess and

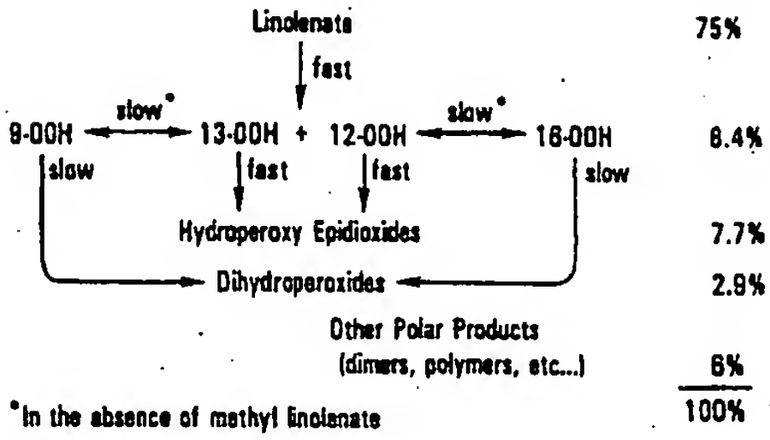


FIG. 10. Secondary oxidation of methyl linolenate. Percent composition on the right side is based on silicic acid chromatographic separation of a sample of linolenate oxidized to a peroxide value of 1286.[86]

experiments with pure hydroperoxides. In the presence of linolenate, the predominant reaction is cyclization of the 12- and 13-hydroperoxyl radicals (Fig. 6). When pure linolenate hydroperoxides are further oxidized, cyclization can only occur by isomerization of the major 9- and 16-hydroperoxyl radicals to the internal 12- and 13-hydroperoxides. Under these conditions another competing reaction is the formation of dihydroperoxides[86,124] (Fig. 10). This interpretation is supported by the results of Peers *et al.*,[92] showing that cyclization of the 12- and 13-hydroperoxyl radicals is reduced and only monohydroperoxides are formed in the presence of 5% α-tocopherol. In the presence of 10% α-tocopherol, Coxon *et al.*[21] recently demonstrated the selective formation of 9,16-dihydroperoxides during oxidation of methyl linolenate.

The purified 9-hydroperoxide of γ-linolenic acid (obtained enzymatically) was converted to the bicyclic endoperoxide by incubation with di-*tert*-butyl peroxyoxalate to form the corresponding peroxyl radical.[97]. Evidence for the prostaglandin-like structure XVIIB was based on GC–MS of the triol obtained by reduction compared to prostaglandin PGF.

The structural relationship between the endoperoxides from oxidized linolenate and the prostaglandin endoperoxides formed biosynthetically from arachidonic acid have generated considerable interest. Recently, the stereoisomers of XVIIB were isolated by HPLC from the autoxidation products of pure 13-hydroperoxide of α-linolenate and 9-hydroperoxide of γ-linolenate.[89] These bicycloendoperoxides were shown to have mainly *cis* substituents, in contrast to the natural *trans* stereochemistry of prostaglandins derived enzymatically from arachidonic acid.[96] The physiological importance of this difference in stereochemistry between the non-enzymatically and enzymatically produced bicycloendoperoxides has not yet been established.

From methyl linolenate treated with singlet oxygen, we identified small amounts of hydroperoxy bicycloendoperoxides of structure XVIIB as a pair of positional isomers, with the hydroperoxide group on either C-9 or C-16.[87] The 9-hydroperoxide isomer was the same as that identified in autoxidized 13-hydroperoxide of linolenate.[89] Major secondary products that we identified in singlet oxidized methyl linolenate included isomeric mixtures of dihydroperoxides (9,12-, 10,12-, 13,15-, 13,16-, 10,16-, 9,15- and 9,16-), hydroperoxy epidioxides, and hydroperoxy bis-epidioxides.[87] The major portion of the hydroperoxy epidioxides identified were those derived from cyclization of the 12- and 13-hydroperoxyl radicals of linolenate, XA and XB (Fig. 6). The hydroperoxy epidioxides expected from cyclization of the 10- and 15-hydroperoxyl radicals were found only in minor amounts, because they have homoallylic unsaturation and tend to cyclize again to form a mixture of hydroperoxy bis-epidioxides XVIIIA and XVIIIB (Fig. 11). This serial cyclization and the formation of monoepidioxides and bicycloendoperoxides are free radical side reactions of the singlet oxidation, which produces the hydroperoxides that lead to peroxyl radicals that can cyclize rapidly.

Serial cyclization has also been observed by autoxidation of the 15-hydroperoxide of arachidonic acid initiated with di-*tert*-butyl hyponitrite.[70] A mixture of diastereomers was identified with the structure XIX. The bimolecular addition of oxygen to an epidioxyl

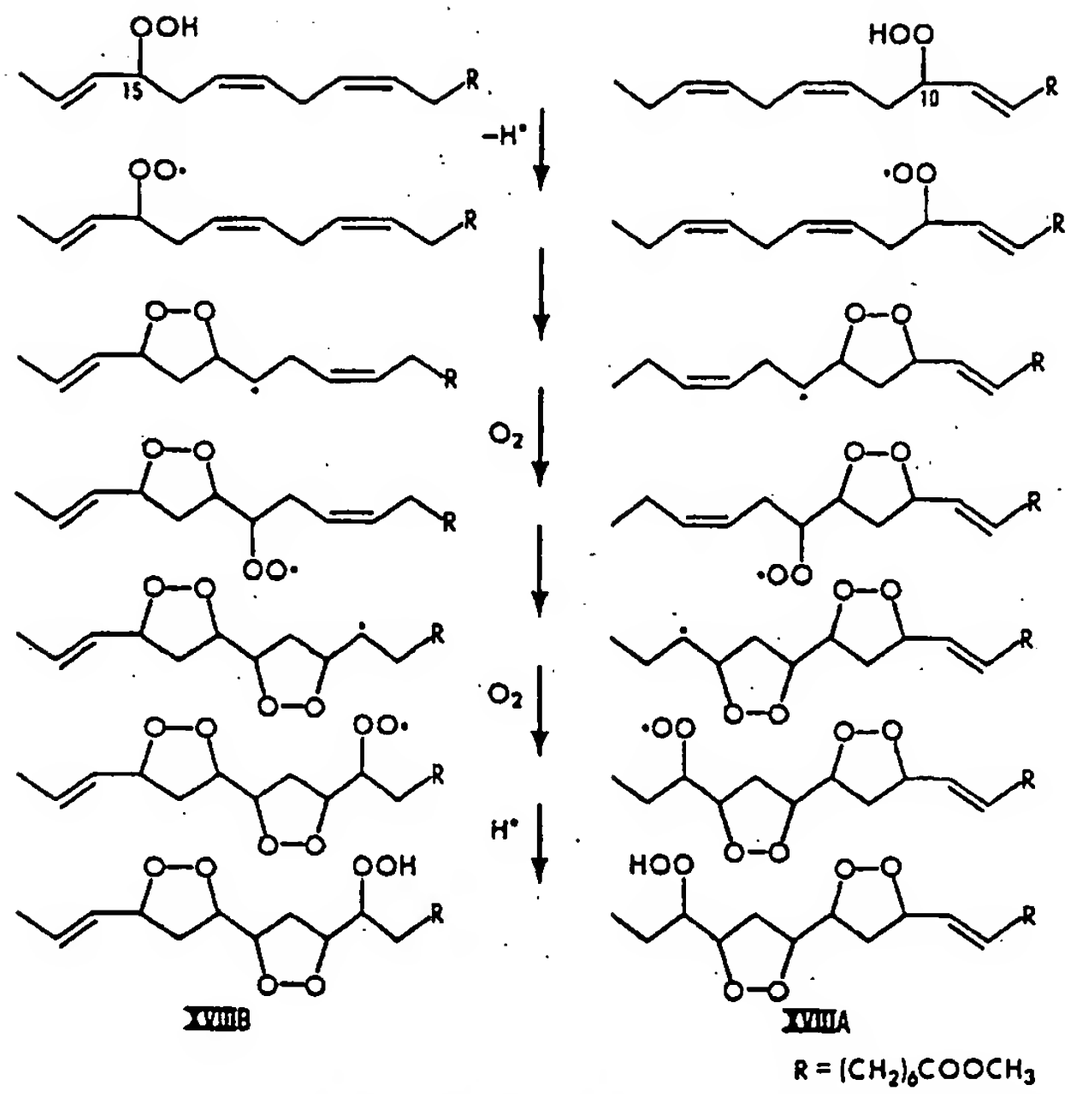
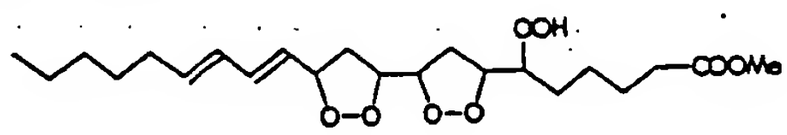


FIG. 11. Formation of hydroperoxy bis-epidioxides from methyl linolenate treated with singlet oxygens.[87]

radical necessary for the formation of XIX was considered to be competitive with bicyclization leading to XVIIB.[70]



XII

The photosensitized oxidation of the mixture of hydroperoxides found in autoxidized methyl linolenate produced five and six-membered cyclic peroxides and hydroperoxy bis-cyclic peroxides, each with one five- and one six-membered ring.[83] The six-membered cyclic peroxides were those expected from 1,4-cycloaddition of singlet oxygen to the conjugated system in 9- and 16-linolenate hydroperoxide isomers after their isomerization to the *trans,trans* configuration (Fig. 12). The bis-cyclic peroxides were formed by 1,4-cycloaddition of singlet oxygen to the conjugated diene system in the hydroperoxy five-membered cyclic peroxides derived from the 12- and 13-hydroperoxide isomers of linolenate (Fig. 13).

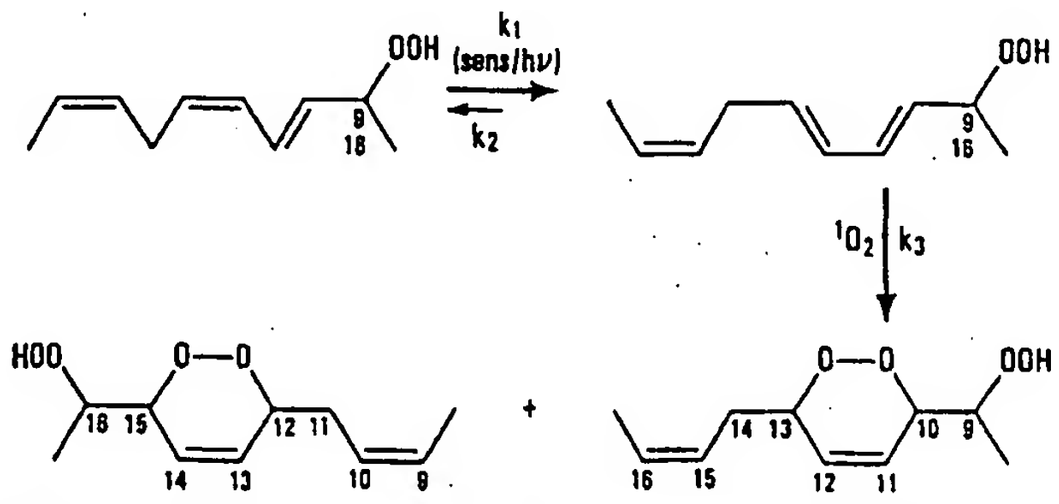


FIG. 12. Scheme for the formation of six-membered hydroperoxy epidioxides from linolenate hydroperoxides (sens = sensitizer).[83]

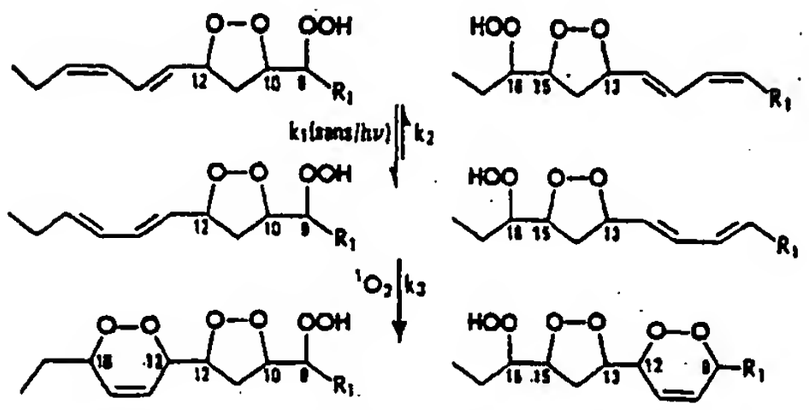


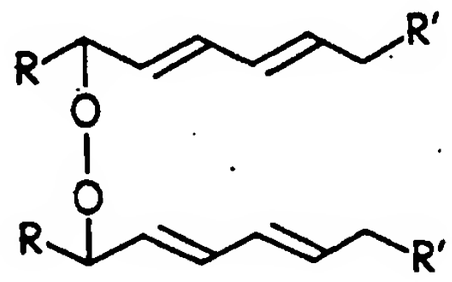
FIG. 13. Scheme for the formation of six/five-membered hydroperoxy bis-epidioxides from linolenate hydroperoxides (sens = sensitizer).[83]

Complex isomeric mixtures of dihydroxy, trihydroxy, tetrahydroxy and pentahydroxy compounds were identified by GC–MS analysis (after reduction and hydrogenation) of the secondary autoxidation products of methyl arachidonate.[126] The corresponding polyhydroxy compounds were isomerically different when the purified monohydroperoxides of methyl arachidonate were oxidized in the presence of Fe(II)–ascorbic acid. The diols were assumed to come from either epoxyhydroperoxides or dihydroperoxides and the triols from either epoxyhydroperoxides or hydroperoxy epidioxides. The tetrahydroxy compounds were assumed to come from a dihydroperoxy bicycloendoperoxide derived from the 15-hydroperoxide of arachidonate. The 5,6,8,9,11-pentahydroxy compound was assumed to come from a hydroperoxy epidioxy bicycloendoperoxide derived from the 11-hydroperoxide of arachidonate.[126] The same pentahydroxy ester was previously shown to come from the bis-epidioxide XIX, which was isolated by HPLC.[70]

B. *High Molecular Weight Products*

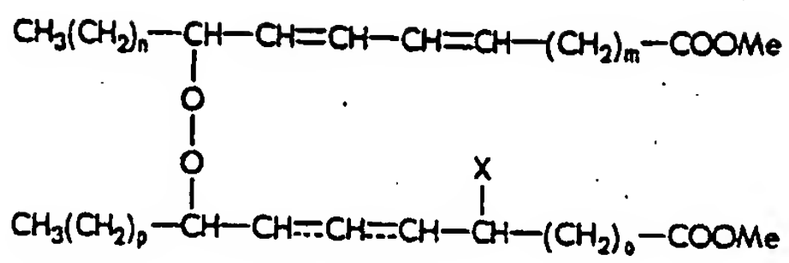
Much work has been reported on the monomeric and volatile decomposition products of lipid oxidation, but information is relatively sparse on the high molecular weight products, which appear to occur in high yields. Although there are indications that these high molecular weight products cause flavor deterioration in oxidized fats, the biological significance of these compounds is unknown.

Dimers of varying polarity and scission acids were the primary products of decomposition of methyl linoleate hydroperoxides stored at 4°C under either oxygen or nitrogen.[68] When linoleate hydroperoxides were decomposed at 210°C in the absence of oxygen, the principal products were dimers that were linked by a carbon–carbon bond and contained 1 mole hydroxyl, 0.5 mole carbonyl and 2 double bonds per mole.[35] Similarly, when methyl linoleate hydroperoxides were decomposed in the presence of an equimolar amount of Fe(II), the major product was a mixture of dimers containing oxygenated groups.[54] Decomposition of the 13-hydroperoxide of methyl linoleate at 38°C in the presence of di-*tert*-butylperoxyoxalate under nitrogen produced a peroxidic dimer of general structure XX.[112] Three isomeric compounds were suggested with peroxidic linkages



XX

between C-9 and C-13, C-9 and C-9′, C-13 and C-13′ or C-9 and C-13′. Similar peroxidic dimeric structures were recently identified from methyl linoleate autoxidized at 30°C.[80] In addition to peroxidic links, the fatty acid components of the dimers were assigned structures with either an allylic hydroperoxide or hydroxy substituent XXI on the basis of reduction studies.



XXI X = OOH/OH

Although these dimers can be isolated from linoleate oxidation products, they include such complex mixtures that they have not been satisfactorily purified and fully identified. The situation is even more complicated with the polymeric products of linolenate hydroperoxides. Our unpublished evidence suggests dimeric compounds consisting of mono-, di-, tri-, tetra- and penta-oxygenated fatty acid units. Because of the difficulty in resolving these polar polymers, their significance in lipid deterioration has yet to be established.

C. *Low Molecular Weight Products*

During the oxidation of lipids, a large multitude of low molecular weight volatile products are formed in small amounts. These compounds can contribute to the flavor of lipid-containing foods at disproportionately low concentrations, even in the range of less than 1 ppm. From the nutritional and biological points of view, the relation of volatile lipid oxidation products to *in vivo* lipid oxidation has generated much interest. Mechanistic concepts for the formation of volatile lipid oxidation products have been reviewed in detail previously.[33] This section will deal only with more recent work, addressing the questions of: How important are secondary lipid oxidation products as precursors of volatile materials? Is malonaldehyde a significant volatile lipid oxidation product? How relevant is the heterolytic cleavage mechanism to the decomposition of primary and secondary lipid oxidation products?

From the thermal decomposition of hydroperoxy cyclic peroxides from methyl linoleate[47] and linolenate,[45] we identified most of the same volatile cleavage products as formed from the corresponding monohydroperoxides.[44] The main volatile products can be explained by cleavage between the epidioxide ring and the alkoxy group. Our evidence also supports oxygen–oxygen cleavage of the epidioxide ring, which forms methyl ketones as unique products. Our results with the hydroperoxy cyclic peroxides of methyl linolenate were confirmed to a large extent by Peers *et al.*,[93] who also identified 2,3-pentanedione from the 16-hydroperoxy epidioxide of linolenate and explained its formation by oxygen–oxygen cleavage of the epidioxide ring. The same cleavage explains the formation of methyl 13-oxo-9,11-tridecadienoate observed in both studies.[45,93]

Thermal fragmentation of hydroperoxy bis-epidioxides XVIIIA and XVIIIB followed the same pattern as the monocyclic peroxides in showing important cleavage between the ring and the hydroperoxide-bearing carbon.[45] On the other hand, the hydroperoxy bicycloendoperoxides XVIIB cleave mainly between the hydroperoxide group and the allylic unsaturation[46] (Fig. 14). Thermal cleavage across the endoperoxide explains the formation of methyl 13-oxo-9,11-tridecadienoate from the 16-hydroperoxide isomer and 2,4-heptadienal from the corresponding 9-hydroperoxide isomer. The same cleavage would be expected to produce malonaldehyde,[88,106] but this dialdehyde was not detected under our thermal decomposition conditions. Malonaldehyde was detected, however, as a product of XVIIB under acid decomposition conditions[39] (cf. below).

Dihydroperoxides behaved like monohydroperoxide on thermal decomposition by forming products mainly derived from cleavage on both sides of the carbons bearing the hydroperoxide groups.[46] For example, the 9,16-dihydroperoxide XXII of linolenate was thermally decomposed into methyl 9-oxononanoate and propanal as main products from internal cleavages A and B. Methyl octanoate and ethane, formed in smaller amounts, are the products expected from cleavages C and D on the other side of the hydroperoxide

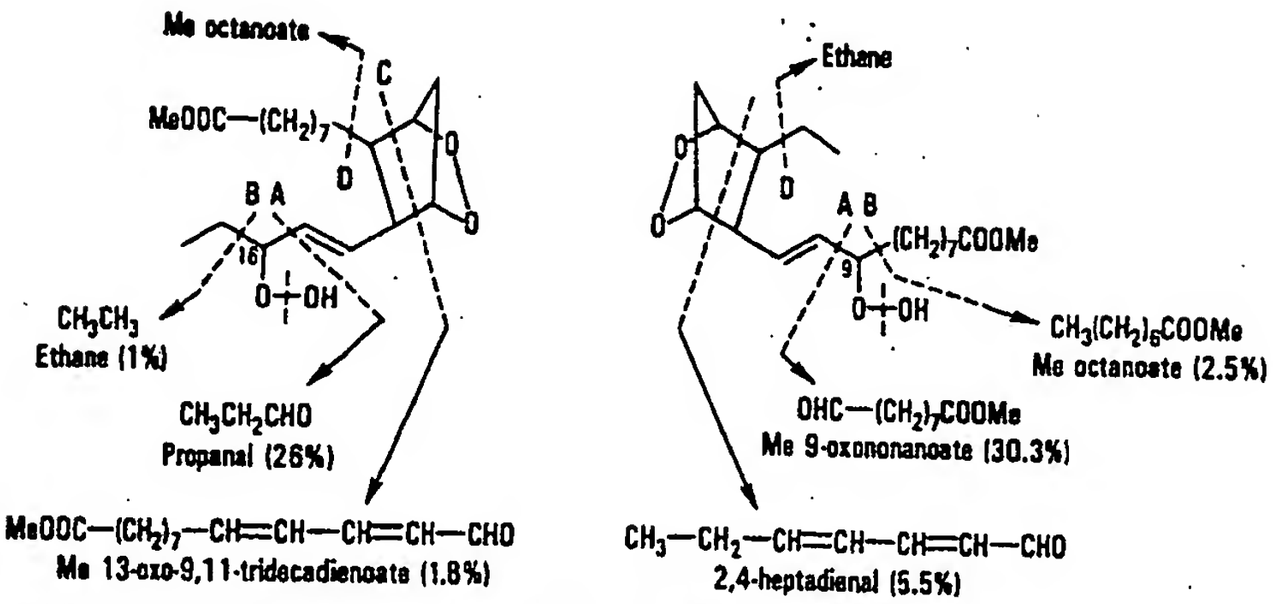
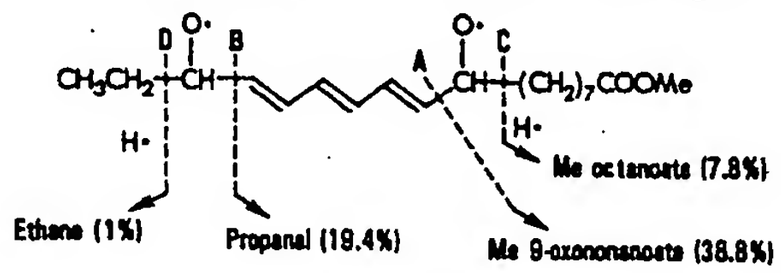
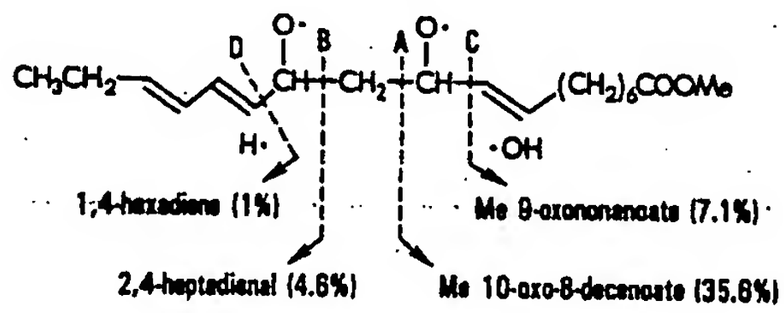


FIG. 14. Thermal decomposition cleavages of hydroperoxy bicycloendoperoxides of linolenate.[46]

groups. Similarly, the 10,12-dihydroperoxide XXIII forms by thermal cleavage methyl 10-oxo-8-decenoate and 2,4-heptadienal by internal cleavages A and B, and methyl



XXIII



XXIV

9-oxonanoate and 1,4-hexadiene by external cleavages C and D. The same cleavages C and D would be expected to produce malonaldehyde. Although this dialdehyde was not detected by thermal decomposition, it was found among the acid decomposition products[39] (cf. below).

The conditions used above for thermal decomposition (200°C) were expected to be too severe for malonaldehyde to survive as a decomposition product from various lipid oxidation compounds. Therefore, we developed a reagent consisting of 5% HCl in methanol (plus trimethyl orthoformate as dehydrating agent) for the decomposition of lipid oxidation products at room temperature. The aldehyde and dialdehyde cleavage products were converted into GC stable dimethyl and tetramethyl acetals, respectively.[39,46] Under these acid conditions, the five-membered hydroperoxy cyclic peroxides were found to be rich sources of malonaldehyde, varying from 0.5% for the epidioxides of linolenate (XA, XB, Fig. 6) to 5% for the epidioxides of linoleate (Fig. 8). The hydroperoxy bisepidioxides XVIIIA and XVIIIB (Fig. 11) produced 1% malonaldehyde, compared to 0.8% from the hydroperoxy bicycloendoperoxides XVIIB. Finally, the 10,12- and 13,15-dihydroperoxides of linolenate produced 1% malonaldehyde and, as expected, the 9,16- and 10,16-dihydroperoxides produced none. No correlation was found between the TBA values obtained for these oxidation products and the GC analyses for malonaldehyde as the tetramethyl acetals. We therefore confirmed a large body of evidence indicating that the TBA method is not specific for malonaldehyde.[39]

By decomposing the monohydroperoxides of methyl linoleate with HCl–methanol, we obtained a mixture of 3-nonenal and methyl 9-oxononanoate expected from the

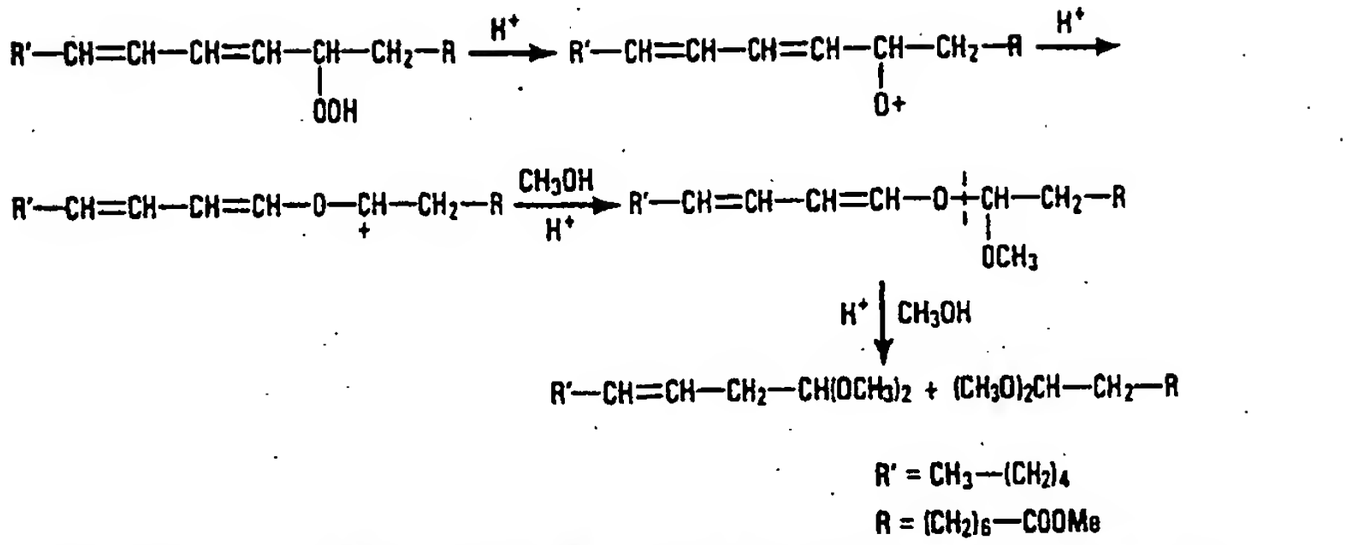


FIG. 15. Mechanism for the heterolytic cleavage of linoleate hydroperoxides with HCl–methanol.[46]

9-hydroperoxide, and a mixture of hexanal and methyl 12-oxo-9-dodecenoate expected from the 13-hydroperoxide.[46] These products can be explained by a heterolytic mechanism proceeding by selective cleavage between the hydroperoxide group and the allylic unsaturation (Fig. 15). The products reported from the decomposition of pure isomers of linoleate hydroperoxides with trichloroacetic acid in benzene included hexanal from the 13-isomer and a mixture of hexanal and 2-nonenal from the 9-isomer.[60] The formation of hexanal from the 9-isomer may indicate isomerization to the 13-isomer with the strong acid reagent. The products reported from decomposition of pure isomeric linoleate hydroperoxides with BF_3 in ether[55] were the same as those we identified from the 9- and 13-hydroperoxide mixture in autoxidized methyl linoleate.[46] In contrast to these studies, a more complex mixture of volatile products was formed by thermal decomposition of linoleate hydroperoxides.[44]

By treating the mixture of 9- and 16-hydroperoxy epidioxides of methyl linolenate (XA and XB, Fig. 6) with HCl–methanol, we obtained a mixture of aldehydes that can be explained by the scheme in Fig. 16.[46] The formation of malonaldehyde (as the tetramethyl acetal) was explained by a heterolytic mechanism involving the formation of an ether intermediate allylic to the double bond.[39] Our identification of the dimethyl acetals of 2,4-heptadienal from XB and methyl 13-oxo-9,11-tridecadienate from XA supports oxygen–oxygen cleavage C (Fig. 16). The acid cleavage products from the hydroperoxy bicycloendoperoxides XVIIB of linolenate include propanal from the 16-isomer and methyl 9-oxononanoate from the 9-isomer.[46] These products are those expected from heterolytic ruptures between the hydroperoxides and the allylic unsaturation (Fig. 17). On the other hand, malonaldehyde formation involves carbon–carbon cleavage across the bicyclic ring.

On one hand, the thermal homolytic mechanism[33] involves a vinyl radical that may be regarded as an unlikely intermediate because of its instability. On the other hand, the acid heterolytic mechanism does not produce many of the volatile materials found from

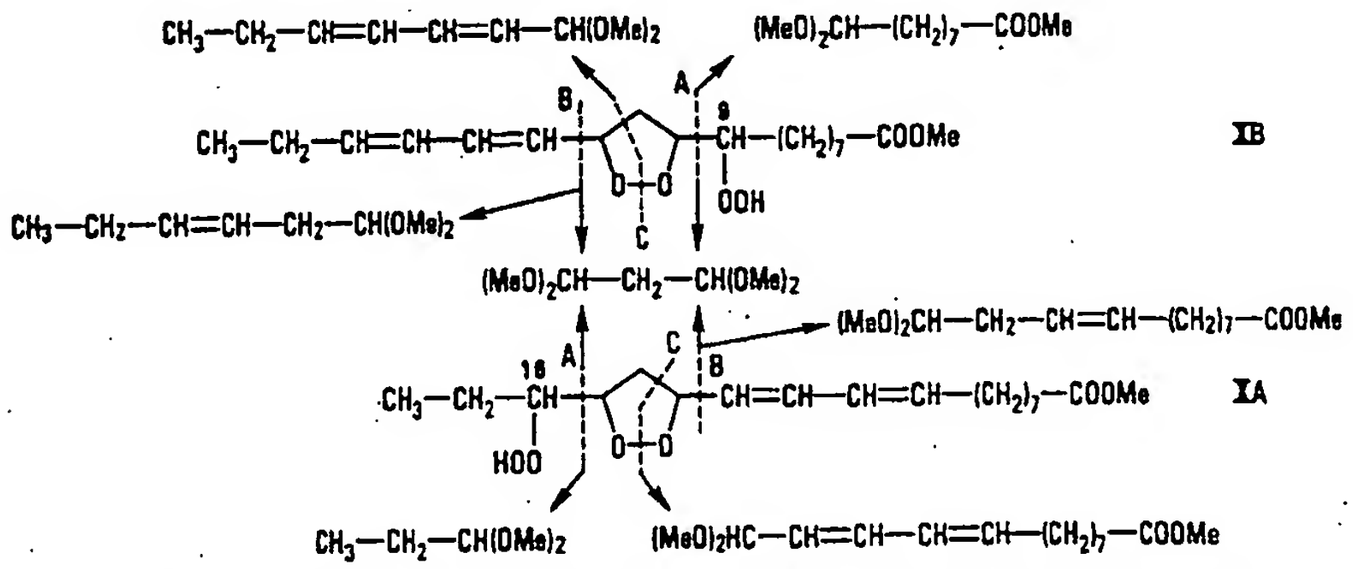


FIG. 16. Heterolytic cleavages of hydroperoxy epidioxides of linolenate with HCl–methanol.

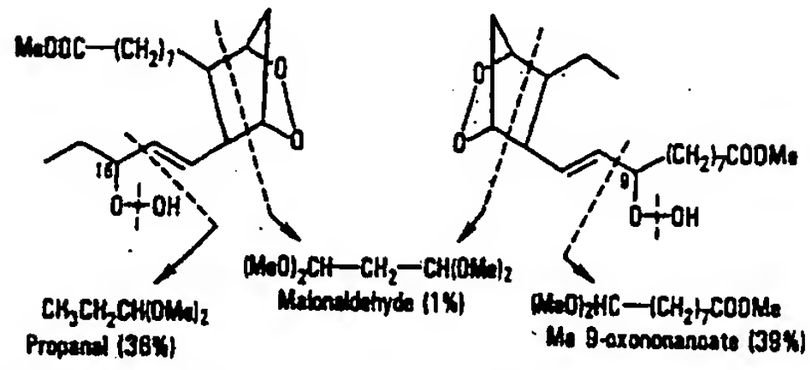


FIG. 17. Heterolytic cleavages of hydroperoxy bicycloendoperoxides of linolenate with HCl–methanol.[46]

hydroperoxides and secondary oxidation products including hydrocarbons, alcohols, ketones and esters.[33] A "mixed" homolytic–heterolytic mechanism is advanced in Fig. 18 that may explain the formation of cleavage products other than aldehydes and avoids the formation of unfavorable vinylic radicals. Heterolysis on the unsaturated side of the hydroperoxide may occur under neutral conditions because hydroperoxides are reported to have higher acid strengths than the corresponding alcohols.[5] Homolysis by β-scission on the alkyl side of the hydroperoxide would produce 2-alkenals, and the alkyl radical would lead to hydrocarbons, alcohols and lower esters.

IV. BIOLOGICAL AND DIETARY LIPID OXIDATION

Malonaldehyde, respiratory hydrocarbons and aldehydes, especially hydroxy alkenals, have been regarded as important biological breakdown products of lipid oxidation responsible for cell-damaging reactions.[90,109,115] However, there is now evidence to suggest that the biological importance of malonaldehyde may have been greatly exaggerated. There are also questions being raised that the measure of respiratory hydrocarbons may not have a direct bearing on membrane lipid oxidation.

Malonaldehyde has been used frequently as a model lipid oxidation product to study the formation of polymers,[9,17] loss of enzyme activity[16,17] and the fluorescent products with amino acids and proteins,[17] and DNA.[108,115] These cross-linking reactions, forming brown pigments and fluorescence, have been related to aging or tissue injuries.[26] The mutagenicity from the reaction with DNA has been attributed to malonaldehyde.[81,113,114] However, recent work showed that the mutagenic activity of most earlier preparations of malonaldehyde, based on acid hydrolysis of its acetal, was due almost entirely to side products such as β-alkoxy acrolein and 3,3-dialkoxy propionaldehyde.[75] When malonaldehyde was synthesized enzymatically, it was found to be without mutagenic activity in the standard *Salmonella*/microsome assay strains.[2]

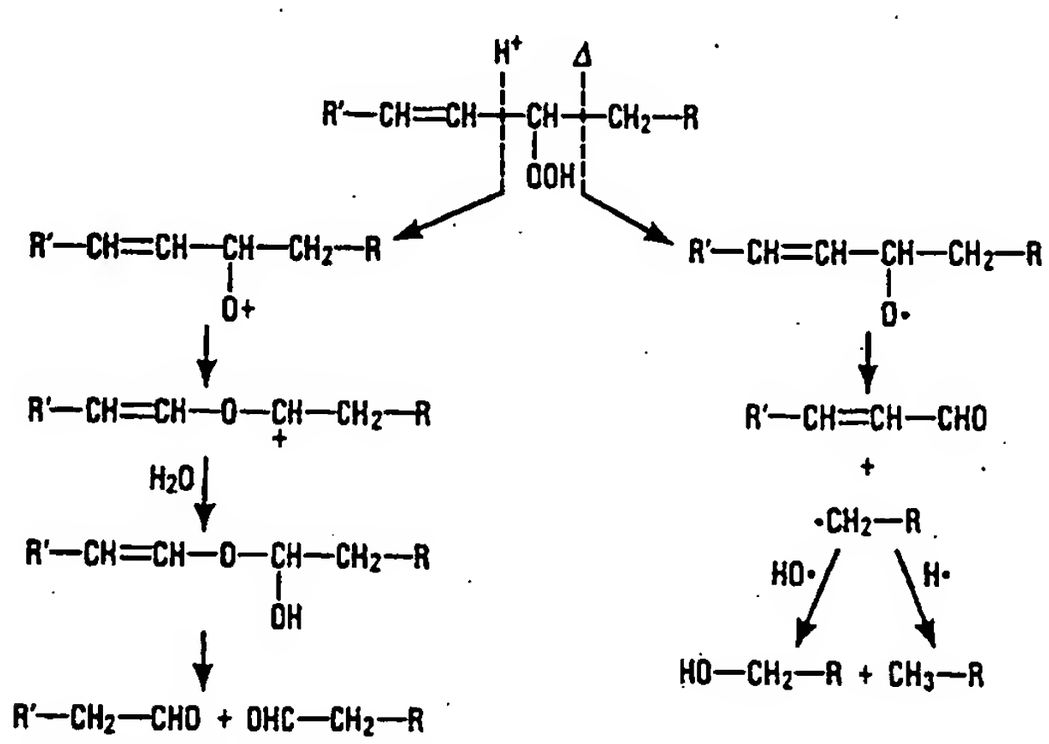


FIG. 18. Homolytic–heterolytic mechanism for decomposition of allylic hydroperoxides.

Many studies implicating malonaldehyde in biological systems were based on the notoriously non-specific TBA test. Our work on the formation of malonaldehyde from various primary and secondary lipid oxidation products showed no relation between the analyses for this dialdehyde and the TBA test.[39] We also recently compared the effect of a wide variety of lipid oxidation products with that of malonaldehyde by determining the fluorescence formed from their interactions with DNA.[51] At low concentrations of oxidation products, fluorescence formation required the presence of metals and ascorbic acid or cysteine. Under these conditions, the relative amount of fluorescence formed from different oxidation products decreased in the order: monohydroperoxides, hydroperoxy epidioxides, dihydroperoxides, hydroperoxy bisepidioxides and bicycloendoperoxides. Malonaldehyde, on the other hand, produced very little fluorescence, and the TBA values did not correlate with fluorescence. We therefore concluded that, in our model reaction system, DNA forms fluorescent products by the breakdown of lipid oxidation products in the presence of metals and ascorbic acid into reactive materials *other than malonaldehyde.*

In vivo lipid oxidation is known to be inhibited by selenium glutathione peroxidase, which reduces hydroperoxides to the stable hydroxy derivatives.[116] This enzyme system is therefore considered among the important body defense mechanisms against lipid oxidation. Although this enzyme apparently does not reduce hydroperoxides in the membrane,[76] it is reported to reduce hydroperoxides in the gastrointestinal tract[7] and the liver.[18] The formation of respiratory hydrocarbons in animals is related to the extent of oxidative damage caused by such agents as ozone, carbon tetrachloride, ethanol, nitrogen dioxide and iron compounds.[116] On the other hand, vitamin E and other antioxidants, and selenium decrease the formation of respiratory hydrocarbons. Oxidative damage by exposure to atmospheric pollutants and hydroperoxides in rancid foods may also be related to a decrease in the activity of the glutathione peroxidase system.[115] In one report, the formation of expired pentane was shown to increase significantly in rats after exhaustive swimming.[57] In another report, respiratory pentane in rats was shown to originate from the action of intestinal bacteria on linoleate hydroperoxides and not from membrane lipid peroxidation.[58] Therefore, the formation of respiratory hydrocarbons from exposure to pro-oxidant agents such as hydroperoxides in rancid fats may involve an entirely different mechanism than that from exhaustive exercise. There are compelling reasons for pursuing more research in this field to resolve important questions on the relevance of volatile lipid oxidation products to biological effects in the cell.

A recent review by Ames[1] presented evidence to suggest that rancid fats may be ingested in appreciable amounts, and that they may contain mutagens, promoters and carcinogens, which may pose a cancer risk in the colon and digestive tract. Dietary antioxidants were also suggested as important anticarcinogens. Among a series of letters to the editors of *Science* debating Ames' article, Hunter[66] claimed that ingestion of rancid fats is not significant because of their unpalatable nature, and that the chemical changes occurring in a fat during deep-fat frying may not be extensive under actual conditions because of the usual commercial practice of discarding fats after prolonged frying. A series of pure monohydroperoxides, dihydroperoxides, hydroperoxy mono- and bis-cyclic peroxides isolated from highly oxidized methyl linoleate and linolenate (peroxide values 800–3000) were recently tested by the Ames test and found to be weakly mutagenic.[73] Under conditions of actual food use, oxidation is associated with relatively low peroxide values, usually lower than 10. Therefore, the weak mutagenic response obtained with pure lipid oxidation products does not suggest a marked genotoxic potential from the occurrence of these types of compounds in dietary fats.

The potential impairment of the nutritional value of frying fats has been debated for a long time.[3,94,103] Cyclic fatty acid monomers and polymeric materials are among the products of thermal oxidation that have been investigated most extensively. The cyclic monomers are more readily absorbed than the polymers and may be included in body fats along with dietary lipids. Our recent GC analyses revealed relatively small levels of cyclic monomers, ranging from 0.1 to 0.5% in commercial frying oils.[48] On the other hand, the

amount of other oxidation products, determined as polar and non-eluted materials, varied from 1 to 8%. These materials are very complex and assumed to be mainly polymeric in nature. Another recent study presented evidence that substances in thermally oxidized oils promote peroxidative deterioration in rat liver.[67] Fats heated in air at frying temperatures (170–200°C) contain an extremely complex mixture of products of oxidation, polymerization, hydrolysis, fragmentation and cyclization. As many as 136 compounds were partially characterized in a heated cottonseed oil by Artman and Smith.[4] There is little information available, however, to indicate if any of this multitude of compounds present any hazard in our modern diet.

V. CONCLUSIONS

The profound physiological properties of the lipid oxidation products discussed in this review have led to research on a wide assortment of biological systems in which peroxides and free radicals are implicated. Much attention has been given to the effect of lipid hydroperoxides and their products of decomposition on damage to biological systems. Malonaldehyde has been considered among the most important lipid secondary oxidation products because of its well-documented cross-linking properties with amino group of proteins, enzymes and DNA. However, the importance of malonaldehyde may have been exaggerated because too many studies implicating this dialdehyde were based on the non-specific TBA test known to give a positive test with many other oxidation and interaction products. A multitude of secondary lipid oxidation products other than malonaldehyde have difunctional structures that may also cross-link amino groups to give fluorescent materials. A more direct causal relationship needs to be established between some of the known lipid oxidation products that have been described in this paper and their biological effects. A better understanding of these effects will provide a better basis for controlling the course of diseases related to peroxides and free radicals.

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